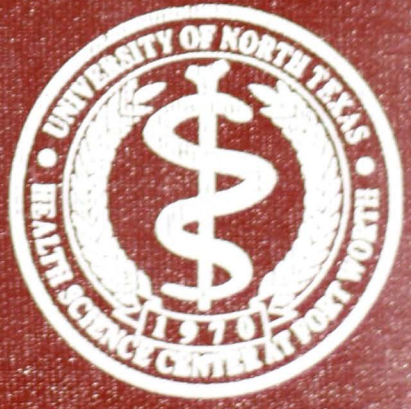
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The current project sought to characterize the interaction of neural and local mechanisms of skeletal muscle blood flow control through *exogenous* and *endogenous* α-adrenoreceptor activation. We hypothesized that α_1- and α_2-adrenoreceptors in the human leg would exhibit differential distribution and responsiveness, and that unilateral knee-extensor exercise would attenuate α-adrenoreceptor-mediated vasoconstriction in an intensity-dependant manner. We also hypothesized that carotid baroreflex (CBR)-mediated sympathoexcitation would provoke less vasoconstriction during exercise than at rest. Intra-arterial infusion of phenylephrine (PE, α_1-agonist) or BHT-933 (α_2-agonist) reduced femoral blood flow (FBF) by approximately 60% at rest, but during exercise (27W) the degree of vasoconstriction evoked by PE and BHT was significantly reduced. During ramped (7W-37W) exercise, BHT did not reduce FBF at any intensity, while some degree of PE-induced vasoconstriction was evident at all but the highest exercise intensity. Using sinusoidal neck pressure, CBR-mediated changes in heart rate (HR), arterial blood pressure (ABP) muscle sympathetic nerve activity (MSNA), FBF, and tissue oxygenation (TO_m) were seen at rest. During 7W exercise, CBR-mediated control of ABP, FBF, and TO_m was attenuated. We conclude that exercise attenuates α-adrenergic responsiveness to exogenous and endogenous activation to ensure sufficient muscle blood flow while maintaining systemic ABP homeostasis.

INTERACTION OF NEURAL AND LOCAL MECHANISMS IN THE CONTROL OF SKELETAL MUSCLE BLOOD FLOW

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DISSERTATION

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LIST OF ABBREVIATIONS

ABP	Arterial blood pressure	NE	Norepinephrine
BHT	BHT-933	NEL	Non-exercising leg
Ca^{++}	Calcium	NIR	Near-infrared
CBR	Carotid baroreflex	NO	Nitric oxide
COH	Coherence	NP	Neck pressure
CP	Chamber pressure	NS	Neck suction
DAG	Diacylglycerol	PE	Phenylephrine
EL	Exercising leg	PKC	Protein kinase C
FAD	Femoral artery diameter	PLC	Phospholipase C
FBF	Femoral blood flow	SR	Sarcoplasmic reticulum
FBV	Femoral blood velocity	IP_3	Inositol trisphosphate
FVC	Femoral vascular conductance	PIP_2	Phosphotidylinositol bisphosphate
HR	Heart rate	RRI	R-R Interval
LF	Low frequency	TFG	Transfer function gain
MAP	Mean arterial pressure	TO_m	Muscle tissue oxygenation
MSNA	Muscle sympathetic nerve activity		

CHAPTER I

INTRODUCTION

Historical perspective.

In 1628, William Harvey first observed that the human circulatory system is organized as a continuous circuit (Harvey, 1628), a finding which has resulted in almost 400 years of fruitful scientific research in the area of cardiovascular physiology. The specific role of the nervous system as a controller of the blood vessels was established by Claude Bernard, who demonstrated vasodilation in the rabbit ear when the ipsilateral sympathetic nerve from the cervical ganglia was severed (Bernard, 1858). This landmark study inspired many specific areas of research regarding control of the vasculature, with a progression of subsequent discoveries regarding specific neurotransmitters (Loewi, 1921), mechanisms of humoral (Oliver and Schäfer, 1894), myogenic (Bayliss, 1902), and metabolic (Cohnheim, 1872) control, and more recently signal transduction pathways (Itoh, 1991; Laughlin & Korzick, 2001). However, much still remains unknown regarding the integration of control mechanisms which collectively govern the peripheral circulation.

Mechanisms of peripheral vascular control in humans.

The peripheral vasculature is an exceptionally dynamic network, continually exposed to the milieu of circulating vasoactive substances as well as constant neural outflow directed towards the smooth muscle surrounding the vessels. These neural and humoral factors act together to vary the degree of vascular smooth muscle "tone", which ultimately determines the diameter of the blood vessel. Since blood flow is the product of vessel diameter and blood velocity, the degree of vascular tone is a key determinant of "downstream" tissue perfusion. Changes in vessel diameter also influence systemic arterial pressure (ABP) according to Ohm's law; *mean arterial pressure = cardiac output / total vascular conductance*. Thus, tight regulation of these resistance vessels is crucial to ensure concomitant delivery of blood to the tissue and adequate systemic ABP.

Vascular tone is regulated by three principal control mechanisms; neural, humoral, and myogenic. It is well-known that *neural* sympathetic activation of the vascular smooth muscle is achieved through the release of norepinephrine (NE), which binds to membrane-bound post-junctional α-adrenergic receptors on the vascular smooth muscle, inducing vasoconstriction. In addition, the vascular smooth muscle is influenced by circulating *humoral* factors, which work through pharmaco-mechanical coupling to promote contraction or relaxation. Vascular smooth muscle tone is also controlled by an inherent constriction in response to stretch, normalizing changes in pressure across the vessel wall via the *myogenic* mechanism. Collectively, these regulatory factors maintain a relatively constant blood flow to the resting tissue. However, during conditions such as exercise, compensatory changes in the neural and humoral inputs to the vascular smooth

muscle are modulated by vasoactive waste products from the exercising muscle tissue. The addition of a metabolic component to the underlying neural and humoral control mechanisms results in a net increase in vascular conductance that is coordinated with the control of systemic ABP. However, details regarding this interaction between local metabolic and systemic vascular control mechanisms are not well known.

Regulation of the vasculature during exercise.

The practical importance of a strict regulation over the peripheral vessels becomes apparent during exercise, an event which may significantly challenge cardiovascular homeostasis. At the onset of exercise, the sympathetic nervous system produces several cardiovascular adjustments, including increases in ABP, heart rate (HR), and regional vascular resistance (Saltin *et al.*, 1998). These adjustments are required to match oxygen delivery to the metabolic demands of exercising skeletal muscle and prevent perfusion mismatch (Laughlin, 1996). During exercise, accumulation of the metabolic byproducts of skeletal muscle contraction activate chemically-sensitive afferent fibers to initiate efferent muscle sympathetic nerve activity (MSNA), a phenomenon commonly referred to as the muscle metaboreflex (McCloskey & Mitchell, 1972). Metabolites also diffuse into the interstitial space and resistance arterioles, directly causing vasodilation through an interaction with the vascular smooth muscle. However, the vascular receptors influenced by these metabolites and the potential impact of these metabolites on sympathetically-mediated vasoconstriction have not been elucidated. *Thus, two distinct factors must be considered; the interactions by which local metabolites are directly*

changing vascular conductance, and how these metabolites may alter the vascular response to neural sympathetic stimulation.

Functional sympatholysis.

In 1962, Remensnyder *et al.* coined the term "functional sympatholysis" to describe the observation that sympathetic vasoconstriction in active skeletal muscle may be overridden by local control factors (Remensnyder, 1962). Since that time, several studies have reported that sympathetic vasoconstriction is well preserved in the exercising muscle (Secher *et al.*, 1977; O'Leary *et al.*, 1991), while others have observed an attenuation of sympathetic activation by the local metabolites produced during exercise (Richardson *et al.*, 1995; Hansen *et al.*, 1996; Hansen *et al.*, 2000b; Rosenmeier *et al.*, 2003). These seemingly conflicting results are not mutually exclusive, since metabolic blunting of sympathetic vasoconstriction does not exclude residual sympathetic effects, but rather implies less vasoconstriction than would be expected for a given sympathetic input. Nevertheless, the underlying mechanisms of sympatholysis are incompletely understood, and remain a topic of many ongoing investigations.

α-adrenoceptors and exercise.

We hypothesize that the common link between neural sympathetic and local metabolic control of skeletal muscle blood flow is the post-junctional α-adrenergic adrenoreceptors. Vasoconstriction of the skeletal muscle vasculature via sympathetic activation is achieved through release of NE, which binds to post-junctional α-adrenergic receptors of the

vascular smooth muscle. Signal transduction of α-adrenoreceptors is dependant upon the subtype (figure 1). The α_1-adrenoreceptors are coupled to $G_{q/11}$ membrane proteins, which stimulate phospholipase C (PLC) activity, promoting hydrolysis of phosphatidylinositol bisphosphate (PIP_2) to produce inositol trisphosphate (IP_3) and diacylglycerol (DAG). These molecules act as second messengers mediating intracellular calcium (Ca^{++}) release from the sarcoplasmic reticulum (SR) and activating protein kinase C (PKC), which increases influx of extracellular Ca^{++} (Hieble *et al.*, 1995; Guimaraes & Moura, 2001). Less is known about the vascular α_2-adrenoreceptors, although it appears the $G_{i/o}$-protein is activated to promote Ca^{++} availability and subsequent vasoconstriction without PLC activation (Docherty, 1998).

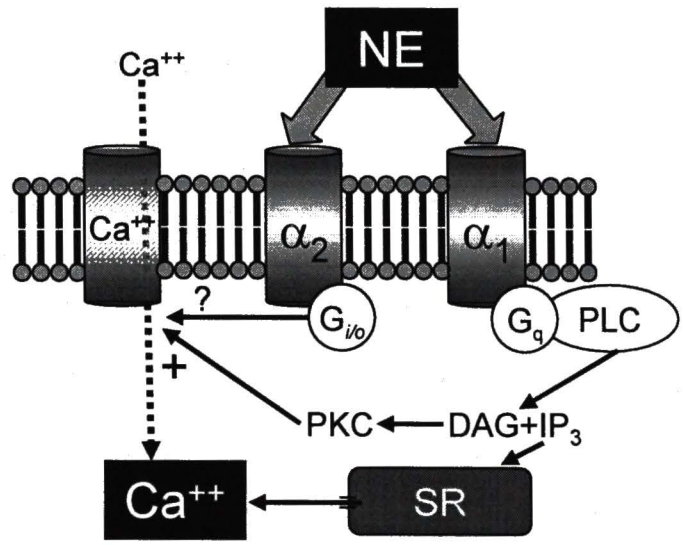

FIGURE 1: Simplified model of adrenergic signal transduction.

The α-adrenoreceptors are anatomically located in a position that is simultaneously exposed to sympathetic input and exercise-induced metabolites, and are logically a putative site of sympatholysis. Indeed, several recent lines of evidence point to inhibition of post-junctional adrenergic signaling via metabolites from active skeletal muscle. Using reflex increases in MSNA, direct sympathetic neural stimulation, and

intra-arterial directly acting α-agonists, recent studies in animals (Thomas *et al.*, 1994; Buckwalter & Clifford, 2001; Ruble *et al.*, 2002) and humans (Hansen *et al.*, 1999; Tschakovsky *et al.*, 2002; Dinenno & Joyner, 2003; Rosenmeier *et al.*, 2003) have all demonstrated a reduction in α-adrenoreceptor responsiveness during exercise. Tschakovsky *et al.* (2002) administered intra-arterial tyramine to the forearm, provoking local endogenous norepinephrine (NE) release from the presynaptic nerve terminals at rest and during rhythmic handgrip exercise. Compared to rest, the vascular response to endogenous NE release was attenuated with exercise in an intensity-dependent manner. Similar results were reported in a parallel animal study with a dynamic exercise protocol (Ruble *et al.*, 2002). These and other studies have identified diminution in post-junctional α-adrenoreceptor responsiveness as an important factor in the modulation of skeletal muscle blood flow during exercise.

A wide array of factors contributing to the observed alteration in vascular responsiveness have been proposed, including vasoactive substances such as adenosine (Laughlin *et al.*, 1989; Saltin *et al.*, 1998; Radegran & Hellsten, 2000), prostaglandins and thromboxanes (Karamouzis *et al.*, 2001), and the indirect effect of increased muscle temperature (Cooke *et al.*, 1984), hypoxia (Hansen *et al.*, 2000a), and acidosis (McGillivray-Anderson & Faber, 1990). These factors are not mutually exclusive. In several different protocols in both rodents and human skeletal muscle, production of nitric oxide (NO) has also been identified as an important metabolic event, since lack of skeletal muscle NO synthase or pharmacologic blockade of NO production is accompanied by loss of metabolic inhibition of sympathetic vasoconstriction (Thomas &

Victor, 1998; Sander *et al.*, 2000; Chavoshan *et al.*, 2002; Thomas *et al.*, 2003). However, the specific interaction of NO with α-adrenoreceptors in the human forearm vasculature has recently been called into question (Dinenno & Joyner, 2003).

Few studies have considered molecular aspects of the interaction between the above metabolites and the various vasoconstrictor pathways in the vascular smooth muscle. In the dog hind limb, exercise attenuates vasoconstriction produced by administration of α-adrenergic agonists (Buckwalter *et al.*, 2001) but not exogenous application of vasopressin (Buckwalter, personal communication). Since α_1-adrenergic and vasopressin (V_1) membrane-bound receptors share a common G_q-protein-mediated signal transduction pathway, this finding identifies a metabolic attenuation that is specific for α-adrenoreceptors. Others have demonstrated that systemic hypoxia (Dinenno *et al.*, 2003), exogenous adenosine infusion (Tschakovsky *et al.*, 2002), and local NO synthase blockade (Dinenno & Joyner, 2003) do not attenuate α-adrenergic vasoconstriction during exercise. Thus, while it seems the metabolic influence on local blood flow is not entirely indiscriminate, the interaction of metabolites with specific membrane-bound receptors and associated signal transduction pathways are not well understood.

α-adrenoreceptor subtypes. Alpha adrenoreceptors are classified according to subtypes, based on pharmacologic binding studies. The primary post-junctional vascular smooth muscle subtypes are α_1 and α_2, and it is generally accepted that these subtypes are distributed heterogeneously within the arterial vessels of the skeletal muscle circulation. Current evidence exists in the animal model for a predominance of α_2

receptors in the small, nutrient arterioles, and a combination of α_1 and α_2 receptor subtypes in the larger, resistance arterioles (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991). In animals it has been postulated that the reflex control of systemic blood pressure is maintained by the upstream resistance arterioles, while control of microvascular blood flow is managed primarily by the smaller nutrient arterioles.

Dinenno *et al.* (2002b) examined the role of α-adrenoreceptor subtypes in the differential control of basal vascular tone in the human forearm using selective α-adrenoreceptor blockade to create "pharmacological sympathectomy". They observed that the calculated increase in forearm blood flow and forearm vascular conductance after removal of α_2-vasoconstrictor tone was greater than after removal of α_1, with the α_2-adrenoreceptors representing ~63% of basal sympathetic tone. These data suggest a small but significant differentiation of α-adrenoreceptor subtypes in the control of basal vascular tone, and also raises the question of whether this differential control might impact skeletal muscle blood flow and ABP regulation during exercise.

α-adrenoreceptor subtypes and exercise.

The functional consequence of the heterogeneous subtype distribution may be seen during exercise, since the α-adrenoreceptors of the microvasculature are in close proximity to the active muscle and thus may be more susceptible to the influence of the local metabolic byproducts produced in the active skeletal muscle. Indeed, in animal studies the sensitivity to metabolites produced during exercise appear greater in the α_2-adrenoreceptor compared to the α_1-adrenoreceptor (Thomas *et al.*, 1994). Isolated vessel

studies suggest a relative insensitivity of the α_1-adrenoreceptor to changes in pH (Medgett *et al.*, 1987; Tateishi & Faber, 1995), hypoxia (McGillivray-Anderson & Faber, 1990), and ischemia (McGillivray-Anderson & Faber, 1991) compared to the α_2-adrenoreceptor. Recently, selective agonists for α_1-adrenoreceptors (PE, phenylephrine) and α_2-adrenoreceptors (clonidine) were applied to resting and exercising (low and high intensity) dog hind limb to consider the differential vascular conductance changes of the receptor subtypes (Buckwalter *et al.*, 2001). Following PE (α_1-agonist), decreases in femoral artery conductance were seen, and the response was attenuated only during heavy exercise. In contrast, attenuation of vascular conductance changes in response to clonidine (α_2-agonist) was observed even at low-intensity exercise. Conversely, Rosenmeier *et al.* (2003) used similar sympathomimetic drugs to demonstrate comparable responses between α_1- and α_2-adrenoreceptor-mediated vasoconstriction in the human forearm at rest, and also reported similar attenuation of α-adrenoreceptor-mediated vasoconstriction between subtypes during handgrip exercise.

These disparate findings have put forth the idea that while α-adrenoreceptor-mediated vasoconstriction may be attenuated to different degrees among receptor subtypes, this relationship may be attributed to differences among species or even the location of the vascular bed under investigation. We suggest that interpretation of these findings must take into account the limitations of animal hind limb and human forearm measurements, since recent findings have shown the arm and leg skeletal muscle vasculature in humans may exhibit markedly different hemodynamic and vascular responsiveness to sympathetic nerve stimulation, suggesting both a greater (Pawelczyk &

Levine, 2002) and lesser (Jacob *et al.*, 2000) response to pharmacologic α_1-adrenergic stimulation (PE) in the leg compared to the arm. Therefore, it is possible that these limbs also exhibit a differential vascular response to the metabolic by-products produced during exercise. Exercise models are also an important consideration, since forearm blood flow increases only 6-fold during strenuous handgrip exercise, while leg extension exercise may increase femoral blood flow up to 20-fold (Saltin *et al.*, 1998). The leg indeed represents a "sleeping giant" (Andersen & Saltin, 1985), with a vascular bed that may vasodilate to such a degree that it challenges maintenance of systemic ABP during exercise (Saltin *et al.*, 1998; Volianitis *et al.*, 2003). However, the impact of sympatholysis on the leg vasculature and how this event may influence cardiovascular homeostasis remains unknown.

To our knowledge, no human studies have examined the concept of functional sympatholysis by directly measuring blood flow responses of a large ambulatory muscle group during graded, dynamic exercise. Furthermore, the existence and functional importance of α-adrenoreceptor subtype distribution in the leg vasculature has not been considered in humans. In chapter 2, we present findings from a series of protocols which utilized direct femoral intra-arterial infusion of selective α-agonists to specifically address the role of the α_1- and α_2-adrenoreceptors in the control of skeletal muscle blood flow at rest and during various levels of dynamic leg exercise.

Interaction of neural reflex and local metabolic control mechanisms.

While the role of metabolic vasodilation as a means of ensuring ample oxygen delivery to the exercising tissue is well-recognized, the effect of this event on reflex control of systemic ABP is not well known. The importance of local metabolic events on ABP regulation is underscored by the fact that changes in vascular conductance in the skeletal muscle vascular bed produce more pronounced systemic effects as blood flow in the exercising muscle increases (O'Leary, 1991; Buckwalter & Clifford, 2001), making the skeletal muscle vascular bed more hemodynamically significant to overall cardiovascular homeostasis. The impact of metabolic vasodilation is of particular interest in the human leg vasculature, when muscle blood flow can increase up to 100-fold during intense exercise (Saltin *et al.*, 1998). Thus, effective reflex control of the muscle vasculature is crucial to ensure adequate tissue perfusion is achieved without sacrificing systemic ABP. However, the relationship between reflex and local mechanisms of blood flow control in humans remain unclear.

Experimentally, the neural control of vasomotion may be evaluated using conditions that alter sympathetic nerve activity, which will cause a associated change in vascular conductance of both resting and exercising muscle (Seals, 1989). Acute changes in carotid sinus transmural pressure are known to evoke changes in MSNA (Fadel *et al.*, 2001b) and vascular conductance (Ogoh *et al.*, 2002; Keller *et al.*, 2003). In the resting skeletal muscle, reflex sympathetic activation produces vasoconstriction (Seals, 1989). Thus, it appears that the carotid baroreceptors contribute directly to the neural control of vasomotion and skeletal muscle blood flow through alterations in sympathetic outflow,

both at rest and during exercise. However, the functional consequence of a *reflex* change in sympathetic activity in the exercising skeletal muscle has become a subject of considerable debate.

During exercise, the carotid baroreceptors are exposed to perpetual fluctuations in ABP, and so must provide adequate changes in efferent activity on a beat-to-beat basis. Previous studies have established that carotid baroreflex (CBR) control of R-R interval (RRI) (Eckberg, 1977) and ABP (Bevegard & Shepherd, 1966; Potts *et al.*, 1993) is preserved from rest to exercise in humans. More recently, CBR control of MSNA (Fadel *et al.*, 2001a) and leg vascular conductance (Keller *et al.*, 2003) during exercise have also been demonstrated. Using the static variable pressure neck chamber technique, these studies have elegantly evaluated the degree of cardiac and hemodynamic responsiveness to a single, static pulse of neck pressure (NP, CBR unloading) and neck suction (NS, CBR loading) during exercise. However, two important aspects of reflex control during exercise have yet to be considered; the role of the CBR in control of the *peripheral circulation*, and the emerging model of evaluating the *dynamic* properties of CBR regulation.

CBR control of the peripheral vasculature.

Studies in animals (Collins *et al.*, 2001) and humans (Keller *et al.*, 2003) have utilized reflex maneuvers to evaluate CBR control of limb blood flow, with equivocal results. In the exercising dog, Collins *et al.* (2001) determined that CBR-mediated changes in vascular conductance in the exercising dog hind limb became greater as exercise intensity

increased. Using single bouts of NP and NS in humans, Keller *et al.* (2003) identified attenuation of CBR-mediated changes in vascular conductance in the exercising leg during unilateral knee extension exercise. However, in both studies vascular conductance calculations are based on blood flow measurements taken in large, conduit vessels to estimate limb blood flow, which includes vessels supplying muscle, skin, and bone. Thus, this measurement does not necessarily reflect blood flow solely in the skeletal muscle microcirculation, where blood flow is heterogeneous and influenced by multiple control mechanisms. Recently, near-infrared (NIR) spectroscopy has been utilized for determination of skeletal muscle tissue oxygenation (TO_m) in conjunction with established blood flow measurements to provide an index of oxygen delivery to the microcirculation, allowing an estimate of microcirculatory blood flow (Boushel & Piantadosi, 2000; Hansen *et al.*, 2000b). We have adopted this methodological approach to extend the established paradigm of efferent CBR control over cardiovascular function to the level of the skeletal muscle microcirculation, where local metabolic influences may directly influence reflex control of blood flow.

Dynamic properties of CBR control.

The CBR is by nature a dynamic system, regulating cardiovascular function through a complicated feedback control system that has intrinsic nonlinearity (Eckberg, 1980b; Zhang *et al.*, 2001). As such, recent evidence suggests alternate models may be more appropriate to evaluate the *dynamic* nature of the CBR (Bernardi *et al.*, 1997; Keyl *et al.*, 2000; Zhang *et al.*, 2001). Unique modeling of dynamic reflex control has been achieved

by providing an oscillating input to the CBR and measuring changes at the end-organ using spectral analysis of end-organ variability. Oscillatory CBR stimulation has been applied in studies investigating respiratory sinus arrhythmia (Keyl *et al.*, 2000) and CBR control of muscle sympathetic outflow (Bath *et al.*, 1981) using neck suction. These studies have demonstrated an apparent "entrainment" of RRI, MSNA, and ABP corresponding to the oscillatory frequency of the neck chamber pressure. However, the effect of oscillating CBR-mediated sympathoexcitation (NP) on ABP regulation and skeletal muscle hemodynamics has not yet been considered. Furthermore, the degree of CBR control of peripheral blood flow during exercise is not well understood.

Repeated oscillations in carotid sinus transmural pressure produced by the neck chamber technique provide the opportunity for unique quantification of CBR reflex control using spectral analysis techniques. Cross-spectral analysis methods are now widely used to evaluate the transfer function of RRI and ABP variability as an index of both static and dynamic baroreflex gain (Clayton *et al.*, 1995; Sleight *et al.*, 1995). Others have utilized spectral power analysis of RRI, ABP, and MSNA in the frequency domain to estimate autonomic balance in response to pharmacologic (Saul *et al.*, 1990; Nakata *et al.*, 1998) and reflex (Bernardi *et al.*, 1997) changes in carotid sinus pressure. However, to our knowledge the spectral analysis technique has not been applied to analyze dynamic CBR modulation of hemodynamic control at the level of the skeletal muscle microcirculation using measures of femoral blood velocity (FBV) and skeletal muscle tissue oxygenation (TO_m). Furthermore, cross-spectral analysis of HR, ABP, MSNA,

FBV, and TO_m for analysis of the temporal relationship between end-organs has not been considered.

The extent of CBR control over the skeletal muscle vasculature of the leg in humans is not well understood. This vascular bed is of particular interest because of an enormous vasodilatory capacity during intense exercise, which may challenge cardiovascular homeostasis. Furthermore, details regarding the dynamic properties of CBR control of cardiovascular function at rest and during exercise are not known. In chapters 3 and 4, we present findings from a series of protocols which sought to determine the effect of dynamic oscillatory neck chamber pressure on peripheral hemodynamics at rest and during moderate-intensity knee extension exercise.

Summary and hypotheses.

The current project sought to characterize the interaction of neural and local mechanisms of skeletal muscle blood flow control through *exogenous* (selective agonist drugs) and *endogenous* (CBR-mediated sympathoexcitation) post-junctional α-adrenoreceptor activation. We hypothesized that α_1- and α_2-adrenoreceptors in the human leg would exhibit differential distribution and responsiveness to selective α-agonist drug administration. Furthermore, we hypothesized that exercise would attenuate α-adrenoreceptor-mediated vasoconstriction due to the influence of metabolites emanating from the exercising muscle tissue, and that this effect would be intensity-dependant. Finally, we hypothesized that carotid baroreflex-mediated sympathoexcitation would provoke less vasoconstriction during exercise than at rest due to a metabolic attenuation of α-adrenoreceptor function, but that some degree of reflex control over the vasculature would persist to ensure adequate control of ABP.

In order to test these hypotheses, we have established the following specific aims:

Specific Aim 1: To test the hypothesis that α_1 and α_2 adrenergic receptor subtype function is related to receptor distribution in the skeletal muscle vasculature (Chapter 2).

Specific Aim 2: To test the hypothesis that α-adrenoreceptor-mediated vasoconstriction induced by sympathomimetic drug infusion is attenuated by the local metabolic influences during graded, dynamic exercise (Chapter 2).

Specific Aim 3: To test the hypothesis that α_1 and α_2-adrenoreceptor subtypes exhibit differential sensitivity to the metabolic byproducts produced during exercise (Chapter 2).

Specific Aim 4: To test the hypothesis that skeletal muscle blood flow and tissue oxygenation are directly controlled by the carotid baroreflex (CBR) (Chapter 3).

Specific Aim 5: To test the hypothesis that dynamic CBR control of skeletal muscle blood flow is attenuated by local metabolic factors during dynamic exercise (Chapter 4).

To address these hypotheses and specific aims, three separate experimental protocols were performed. The findings from these studies are presented in the following chapters, in traditional manuscript format as submitted for publication.

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CHAPTER II

Inhibition of α-Adrenergic Vasoconstriction in Exercising Human Thigh Muscles.

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SUMMARY

The mechanisms underlying metabolic inhibition of sympathetic responses within exercising skeletal muscle remain incompletely understood. The aim of the present study was to test whether α_2-adrenoreceptor-mediated vasoconstriction was more sensitive to metabolic inhibition than α_1- vasoconstriction during dynamic knee-extensor exercise. We studied healthy volunteers using two protocols: 1) Wide dose-ranges of the α-adrenoreceptor agonists phenylephrine (PE, α_1 selective) and BHT-933 (BHT, α_2 selective) were administered intra-arterially at rest and during 27W knee-extensor exercise (n=13); 2) Flow-adjusted doses of PE (0.3 $\mu g.kg^{-1}.l^{-1}.min^{-2}$) and BHT (15 $\mu g.kg^{-1}.l^{-1}.min^{-2}$) were administered at rest and during ramped exercise (7W-37W) (n=10). Ultrasound Doppler and thermodilution techniques provided direct measurements of femoral blood flow (FBF). PE (0.8 $\mu g.kg^{-1}.min^{-1}$) and BHT (40 $\mu g.kg^{-1}.min^{-1}$) produced comparable maximal reductions in FBF at rest (-58±6 vs. -64±4%). Despite increasing the doses, PE (1.6 $\mu g.kg^{-1}.min^{-1}$) and BHT (80 $\mu g.kg^{-1}.min^{-1}$) caused significantly smaller changes in FBF during 27W-exercise (-13±4 vs. -3±5%). During ramped exercise, significant vasoconstriction at lower intensities (7 and 17W) was seen following PE (-16±5 and -16±4%), but not BHT (-2±4 and -4±5%). At the highest intensity (37W), FBF was not significantly changed by either drug. Collectively, these data demonstrate metabolic inhibition of α-adrenergic vasoconstriction in large postural muscles of healthy humans. Both α_1- and α_2-adrenoreceptor agonists produce comparable vasoconstriction in the resting leg, and dynamic thigh exercise attenuates α_1- and α_2-mediated

vasoconstriction similarly. However, α_2-mediated vasoconstriction appears more sensitive to metabolic inhibition, because α_2 is completely inhibited even at low workloads, whereas α_1 becomes progressively inhibited with increasing workloads.

INTRODUCTION

Vascular tone is controlled by interactions of sympathetic nervous and local vascular control mechanisms (Laughlin, 1996). In resting muscle, the importance of sympathetic activity for vascular tone is exemplified by the 50-100% increase in blood flow following sympathetic denervation or α-adrenergic blockade (Delp & Armstrong, 1988; Laughlin, 1996; Delp & Laughlin, 1998; Dinenno *et al.*, 2002b). In exercising muscle, metabolites accumulate and become involved in the control of local blood flow. During exercise the need for dramatic increases in perfusion and oxygen delivery to the contracting units are met by increased cardiac output and redistribution of blood flow. The latter is accomplished primarily by increasing sympathetic vasoconstriction in internal organs as well as resting muscle vascular beds. The increased sympathetic vasoconstrictor stimulus is delivered in parallel to the exercising muscle (Hansen *et al.*, 1994), but the effectiveness of this stimuli in the face of local metabolic vasodilation has been a matter of some controversy. In some models of exercise, evidence of sympathetic vasoconstriction has been reported (Secher *et al.*, 1977; O'Leary, 1991), whereas other studies find evidence for metabolic blunting of sympathetic vasoconstriction in the exercising muscle (Thomas *et al.*, 1994; Richardson *et al.*, 1995; Hansen *et al.*, 1996; Thomas *et al.*, 1998; Sander *et al.*, 2000). These seemingly conflicting results are not

mutually exclusive, since metabolic blunting of sympathetic vasoconstriction does not exclude residual sympathetic effects, but rather implies less vasoconstriction than would be expected for a given sympathetic input.

In recent years there seems to be a growing consensus that metabolic inhibition of sympathetic vasoconstriction is a factor in several models of exercise in both animals and humans. Although the underlying mechanisms are incompletely understood, several lines of evidence point to inhibition of post-junctional adrenergic signaling via metabolites from active skeletal muscle. The α-adrenergic receptors can be divided into the α_1 and α_2 subtypes, each with vasoconstrictor properties *in vivo*. Microvascular preparations have identified α_1-adrenoreceptors primarily in proximal and α_2-adrenoreceptors primarily in the distal vasculature of skeletal muscle (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991). In both isolated microvascular preparations and intact animal models, α_2-mediated vasoconstriction seems more sensitive to metabolic inhibition than α_1-vasoconstriction (Anderson & Faber, 1991; Thomas *et al.*, 1994). However, the role of α-adrenoreceptor subtypes in humans remains unclear.

Previous studies considering the effect of exercise on α-adrenoreceptor-mediated vasoconstriction have been limited to the animal hind limb muscles (Buckwalter *et al.*, 2001), and the human forearm during rhythmic handgrip (Rosenmeier *et al.*, 2003). However, to our knowledge no previous human studies have examined α-adrenergic function in a large postural muscle group such as the quadriceps during graded, dynamic exercise. This is an important consideration, since the leg represents a vascular bed with the potential to vasodilate to such a degree that it may challenge maintenance of systemic

arterial blood pressure during exercise (Saltin *et al.*, 1998; Volianitis *et al.*, 2003), and so may be considered more hemodynamically significant than smaller muscle vascular beds.

The aims of this study were to test the following hypotheses: 1) metabolic inhibition of α-adrenergic vasoconstriction is evident in exercising human thigh muscle, and 2) α_2-mediated vasoconstriction is more sensitive than α_1-mediated vasoconstriction to metabolic inhibition in exercising human thigh muscle. To accomplish this, femoral blood flow was measured directly at rest and during steady-state knee-extensor exercise with superimposed administration of the selective α-receptor subtype agonists phenylephrine (PE, an α_1-agonist) and BHT-933 (BHT, an α_2-agonist).

METHODS

Subjects and general procedures. Seventeen healthy young men (25±1yrs) participated in the present study. Written informed consent was obtained from all participants, and experiments were approved by the local ethics committee of Copenhagen and Frederiksberg and conformed to the Declaration of Helsinki. All studies were performed in a thermoneutral environment, with subjects in a semi-recumbent position (approximately 30 degrees reclined). Heart rate (HR) was recorded from an ECG (ADInstruments), and respiratory excursions from a strain gauge belt (Respitrace, ADInstruments). Blood pressure was determined both non-invasively by automated sphygmomanometry (Dinamap), and invasively (see below). Knee-extensor exercise with one leg was performed at 60 RPM on a modified cycle ergometer as described previously

(Andersen *et al.*, 1985). The knee extensor force and rhythm was recorded via a strain gauge attached to the ergometer lever arm (Customized signal processor, FBJ Industries). All measurements at rest were performed during vascular occlusion of the lower leg (pneumatic cuff below the knee inflated to 280 mmHg). With this procedure, a very large fraction of blood flow measured at the level of the groin is supplying the thigh muscle vascular bed.

Catheterization The femoral artery and vein of the exercising leg were cannulated under local anesthesia (Lidocain, 5 ml, 20 mg/ml). The arterial (Arrow, 20 gauge) and venous (Cook, 18 gauge) catheters were inserted in the proximal direction ~5 cm below the inguinal ligament. The arterial catheter was used for intra-arterial infusions of drugs and phasic invasive blood pressure measurements (calibrated to the mid-axillary level, and blood pressure corrected for pump pressure artifacts during infusions). The venous catheter was used to perform thermodilution measurements of leg blood flow, as described below.

Blood flow measurements

Thermodilution. A sterile tip-thermistor (model 94-030-2.5-Fr, Baxter) was inserted through the femoral venous catheter and positioned 8 cm proximal to the catheter tip (at the level of the inguinal ligament). Another thermistor was placed at the venous catheter inlet and both thermistors were connected to a customized dual temperature signal processor. This allows continuous measurements of blood temperature during constant

infusion of a sterile iced saline solution (around 2-3°C at the venous inlet) administered at 100 ml/min by a modified Harvard pump for periods of 20 seconds. Dilution of this indicator in the vein of the exercising leg causes blood temperature to decrease by 0.5 - 3°C, depending on blood flow. This thermodilution procedure has been described and validated in detail previously and provides very accurate and reproducible measurements of leg blood flow during knee extensor exercise (Andersen & Saltin, 1985). During rest, thermodilution was not used, because the method is unsuited for determination of very low levels of blood flow, as expected during vasoconstrictor infusions. Femoral blood flow (FBF_{TD}) was calculated by the formula:

$$FBF_{TD} = F_{sal} \bullet \frac{C_{bl} \bullet \rho_{bl}}{C_{sal} \bullet \rho_{sal}} \bullet \frac{(t_{bd} - t_{ic})}{(t_{bb} - t_{bd})}$$

where F_{sal} is saline flow; C_{bl} and C_{sal} are specific heats for blood and saline, respectively; ρ_{bl} and ρ_{sal} are specific densities for blood and saline, respetively; t_{bb} and t_{bd} are temperatures of blood before and during the saline infusion, respectively; t_{ic} is the saline infusate temperature, corrected to the level of the catheter tip.

Ultrasound imaging and Doppler. The ultrasound machine (model CFM 800, GE Medical) was equipped with a mechanical sector transducer operating at an imaging frequency of 7.5 MHz. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution is achieved (best theoretical resolution around 0.1 mm). The femoral artery was insonated distal to the inguinal ligament for dynamic recordings of diameter throughout a cardiac cycle. The

maximum diameter (systole) was used for calculation of blood flow, because systole is the phase of blood propulsion in large arteries. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 4.0-6.0 MHz, operated in the high-pulsed repetition frequency mode (4-36 kHz) with a sample volume of 5 mm in depth. Care was taken to avoid aliasing especially during exercise. All blood velocity measurements were obtained with the probe at a constant 46 degree (or in some subjects 50 degree) angle to the femoral artery during simultaneous real time 2D vessel visualization to maintain insonation angle constant and sample volume centered. At all sample points we obtained both diameter of the femoral artery (FAD) and, approximately 20-30 seconds later, an angle-corrected, time- and space-averaged, and intensity-weighted mean blood velocity (V_{mean}) (Echopac Software, GE Medical and PowerLab, ADInstruments). Using FAD and V_{mean}, femoral artery blood flow (FBF_D) was calculated from:

$$FBF_D = V_{mean} \cdot \pi \cdot (FAD/2)^2.$$

The Doppler method has been reported to yield reproducible femoral artery blood flow measurements at rest and during knee-extensor exercise, previously used at up to 70% of maximal work (Radegran, 1997a). We obtained FBF_D both during rest and exercise. However, *a priori* the thermodilution method was chosen as the method of choice during exercise, since variability for the thermodilution method was expected to be less compared to the Doppler method. Thus, for the present study, blood flow values during rest are from ultrasound Doppler measurements, while values during exercise are from

the thermodilution technique. A direct comparison of the results obtained from the two methods during exercise was performed (see results section).

Drugs. Phenylephrine (PE) (Danish county pharmaceutical corporation, SAD) was used as a specific α_1-adrenergic agonist. BHT-933 (BHT) (Sigma-Aldrich, Denmark) was used as a specific α_2-adrenergic agonist. Propranolol (Prop) (Astra Zeneca, Sweden) was used as a non-specific β-adrenergic antagonist. In validation experiments, isoproterenol (Iso) (Danish county pharmaceutical corporation, SAD) was used as a non-specific β-adrenergic agonist, while yohimbine (Sigma-Aldrich, Denmark) was used as an α_2-adrenergic antagonist. Drugs were dissolved and diluted as appropriate with normal saline, except yohimbine, which was diluted with sterile water. Intra-arterial infusion rates ranged from 0.2-6 ml/min.

Experimental Protocols. To address the aims of the present study, four separate protocols were performed. Graphic presentations of the protocols are presented in figure 1. Protocols 1 and 2 were designed to validate the drugs administered for β-adrenergic blockade and α_2-adrenoreceptor activation, respectively. In Protocol 3, wide dose-ranges of the α_1-agonist PE and α_2-agonist BHT were administered at rest and during one level of moderate intensity (27W) exercise. This design allows evaluation of the doses required to obtain a maximal drug response. In Protocol 4, one flow-adjusted dose of PE and BHT was administered at rest and during ramped (7W-37W) exercise. This design allows evaluation of the exercise intensity required to attenuate each drug at a concentration

which was shown to yield sub-maximal effects at rest. Details regarding the specific objectives and measurements for each protocol are described below.

Protocol 1: Validation of α-adrenergic blockade.

β-blockade in the leg was used in the dose-response exercise protocol (protocol 3), because PE previously has been shown to have β-adrenergic agonist properties (Torp *et al.*, 2001). Intra-arterial administration of propranolol (Prop) has previously been shown to produce effective β-blockade in the human forearm after 10 minutes (Johnsson, 1967), and in validation experiments we extended this finding to the leg. The purpose of the present protocol was to validate the assumption that the maintenance dose of propranolol used in protocol 3 would sustain the β-blockade in the leg for several hours. We tested this in nine resting subjects by measuring blood pressure, HR and FBF during challenges with intra-arterial Isoproterenol (Iso) (1, 2, 4, and 8 ng/kg/min, 2-min on each dose) before and after 150 minutes of intra-arterial administration of Prop (2.5 μg/kg/min for 5-min as bolus, followed by 0.125 μg/kg/min as maintenance). This dosing regimen for Prop was identical to that used in protocol 3 of the present study.

Protocol 2: Validation of α_2-adrenergic specificity of BHT-933.

The α-adrenergic receptor subtype specificity of PE has been studied quite extensively. In contrast, the specificity of BHT has only been documented sparingly in humans (Jie *et al.*, 1984). The purpose of this protocol was to validate the assumption that BHT in the dose-range used is specific for α_2-adrenergic receptors in the human leg. We tested this in

7 resting subjects by measuring blood pressure, HR and FBF during intra-arterial BHT (20 and 40 μg/kg/min each for 2-min) and PE (0.4 and 0.8 μg/kg/min each for 2-min) before and during the last 10 minutes of concomitant intra-arterial infusion of the α_2-adrenergic antagonist Yohimbine (5 μg/kg/min for 20 min). If BHT specifically acts as an α_2-adrenergic receptor ligand, we would predict that yohimbine would inhibit the BHT-response without affecting the PE response. All subjects participating in protocol 2 also participated in protocol 1 on the same day, and thus α-adrenergic activation and inhibition was performed during concomitant β-blockade.

Protocol 3: Subtype specific α-adrenergic agonists during rest and exercise.

The purpose of this protocol was two-fold: First, to determine to which degree metabolic inhibition of α-adrenergic vasoconstriction is evident in a large muscle mass during dynamic exercise in humans; Second, to determine whether α_2-vasoconstriction is more sensitive to metabolic inhibition than α_1-vasoconstriction at a given workload. Intra-arterial Prop was used to obtain local β-adrenergic blockade. In 13 subjects, we measured blood pressure, HR, and FBF at rest and during 27W one-leg knee-extensor exercise before and during intra-arterial administration of PE (0.025-1.6 μg/kg/min, 7 doses, 2-min each dose) and BHT (2.5-80 μg/kg/min, 6 doses, 2 min each dose). At rest, the highest dose of PE (1.6 μg/kg/min) and BHT (80 μg/kg/min) were only administered to a subset of the subjects (n=4) due to systemic spillover effects. The wide range of drug doses allowed evaluation of saturation kinetics at rest and during exercise, and post-hoc comparison of doses normalized to flow, i.e. a calculation for similar drug-concentration

in femoral arterial blood despite large changes in pre-drug steady-state blood flow. The two agonists were separated by at least 15 minutes to allow washout and restoration of baseline haemodynamic values, and the sequence of drug application was alternated. In this protocol, only the FBF measurements at rest could be performed during vascular occlusion of the lower leg, because in pilot experiments it was determined that prolonged lower leg ischemia (around 15 minutes was needed for completion of the multiple doses of each drug) was too painful during knee-extensor exercise. During exercise, the first 10 minutes were without measurements to allow ample time for blood flow, HR and MAP to reach steady-state levels (Radegran & Saltin, 1998). The exercise lasted a total of 80 minutes.

In pilot experiments, two prolonged infusions of BHT and PE were performed during supine rest with 60-90 minutes between infusions with no indication of tachyphylaxis towards these two drugs. This is in accordance with previously published results which do not indicate development of tolerance towards intra-arterial administration of these α-agonists within the same study day (Jie *et al.*, 1984; Lembo *et al.*, 1994; Lembo *et al.*, 1997).

Protocol 4: α-adrenergic vasoconstriction during different levels of exercise.

The purpose of this protocol was to determine whether metabolic inhibition of α-adrenergic vasoconstriction in the exercising thigh is dependent on exercise intensity. This protocol was performed without β-adrenergic blockade. In 10 subjects, we measured blood pressure, HR, and FBF at rest and during 7W, 17W, 27W, and 37W one leg knee-

extensor exercise before and during intra-arterial administration of PE (flow-adjusted dosing to 0.3 μg/kg/l, 3-min infusions), and BHT (flow-adjusted dosing to 15 μg/kg/l, 3-min infusions). In a subset of the subjects (n=5), the responses to increased doses of PE (0.6 μg/kg/l) and BHT (30 μg/kg/l) were determined during 7W. This was done to determine if the responses seen during exercise were maximal. The brevity of drug infusions during this protocol allowed FBF measurements both at rest and during exercise to be performed during vascular occlusion of the lower leg. The subjects performed two separate bouts of exercise lasting 75-80 minutes, with 90 minutes rest between bouts. Subjects exercised for 12-15 minutes at each level (7W-37W) using an incremental protocol. Blood flow was allowed to reach steady-state during the first 10 minutes of exercise at each level before measurements and drug administration began. The sequence of drug administration was alternated.

Data Analysis and Statistics. All data underwent analog-to-digital conversion and were sampled at 400Hz, recorded on a PC, and analyzed offline with signal processing software (PowerLab, ADInstruments). Mean arterial pressure was derived from the arterial pressure waveform and the sphygmomanometer readings (MAP = diastolic BP + 1/3 pulse pressure). Femoral vascular conductance (FVC) was calculated by the formula: FVC = FBF / MAP. During the last 30 seconds of each baseline or drug dose FBF_{TD} is averaged over 5-10 seconds; FBF_D over 8 seconds; and MAP, HR over at least 30 seconds. Within group differences were assessed by paired Student's t-test and one- or

two-way ANOVA for repeated measures (i.e., repeated measures for both drug and dose). Post-hoc analysis was performed by Dunnett's procedure for one-way ANOVA, and by Tukey's HSD procedure for two-way ANOVA. The Doppler and thermodilution data obtained during the exercise protocols were compared by paired Student's *t*-tests. Data are expressed as mean ± SEM, except when comparing the Doppler and thermodilution methods, where the mean ± 95% confidence intervals were used. Statistical significance was set at $P<0.05$, adjusted by the Bonferroni method as appropriate.

RESULTS

Lasting β-blockade in the human thigh (protocol 1).

The propranolol bolus and maintenance dose provided virtually complete blockade of isoproterenol-induced vasodilation, even 150 minutes after initiation (fig 2).

α-2 adrenergic specificity of BHT-933 in the human thigh (protocol 2).

The local vasoconstrictor responses to high doses of BHT and PE both caused resting femoral blood flow to decrease from about 250 ml/min to 80 ml/min, a 60 % decrease in flow (fig 3). During concomitant yohimbine administration to provide α_2-blockade, resting blood flow doubled. However, despite this increase in resting flow PE still caused flow to decrease to about 80 ml/min, while the effect of BHT was significantly depressed to accomplish only a 20% decrease in flow (fig 3).

Dose-response relationships for PE and BHT at rest and during 27W exercise (protocol 3).

PE and BHT dose-response at rest. The α-adrenoreceptor agonist dose response relationships at rest and during 27W dynamic knee extensor exercise are presented in figures 4 and 5, respectively. There were no significant changes in blood pressure or heart rate (HR) during infusion of the agonist drugs at rest. FAD diameter decreased following PE administration (8.8±0.2 to 5.6±0.4mm, rest vs. PE 0.8 μg/kg/min, $p<0.01$), while BHT caused a much smaller FAD change (from 8.9±0.2 to 7.8±0.3mm, rest vs. 40 μg/kg/min, $p<0.05$). The change in FAD was significantly greater during PE compared to BHT ($p<0.01$). The highest doses of PE and BHT produced comparable reductions in FBF (-58.5±6.3 vs. -64.4±4.3%, PE 0.8 vs. BHT 40) and FVC (-57.4±6.6 vs. -62.4±4.1%, PE 0.8 vs. BHT 40), with a clear plateau at the highest doses (fig 4).

For higher doses of PE, the decrease in FAD dominated blood flow changes. For example, mean blood velocities during baseline and PE 0.8 μg/kg/min were not significantly different (7.7±0.8 vs. 7.6±1.0 cm/s, p=ns), and despite similar decreases in flow, the mean blood velocity was significantly higher during PE 0.8 μg/kg/min compared to BHT 40 μg/kg/min (7.6±1.0 vs. 2.9±0.4 cm/s, $p<0.05$). We also observed a transient decrease (about 10 mmHg) in blood pressure and sleepiness following the highest dose of BHT, both lasting around 30 minutes with some individual variation.

PE and BHT dose-response during exercise. Moderate intensity knee-extensor exercise (27W) was accompanied by an increase in HR (58±2 to 78±2 beats/min, rest vs. exercise, $p<0.01$) with no significant change in MAP (86±2 to 83±2 mmHg). Exercising FBF reached about 3000 ml/min with no change in FAD (8.9±0.2 vs. 9.0±0.2mm, rest vs. exercise). MAP, HR and FBF remained at steady-state levels throughout each exercise bout.

During 27W exercise, PE infusion produced a small but statistically significant change in FAD (9.1±0.2 vs. 8.7±0.3mm, rest vs. exercise, PE 0.8 μg/kg/min, $p<0.05$), and significantly decreased FBF and FVC (Fig 5a). However, the relative change in flow to the highest dose of PE (0.8 μg/kg/min) was significantly smaller than the response at rest (-58±6 vs. -12±5%, rest vs. exercise, $p<0.01$), while the absolute change tended to be higher (-188±33 vs. -380±141 ml/min, rest vs. exercise, p=ns). During the highest PE doses (PE 0.8 and 1.6 μg/kg/min) we observed significant increases in MAP (9±2 and 15±4 mmHg), with concomitant decreases in HR (-9±1 and -14±2 beats/min). In contrast, BHT infusion during exercise produced no significant changes in MAP, HR, FAD, FBF, or FVC (Fig 5B). The difference in FBF response between PE and BHT did not reach significance at this level of exercise. However, the changes in FVC were significantly larger for the last 3 doses of PE vs. BHT (Fig 5b).

Post-hoc "flow-adjustment" was performed to calculate the drug doses yielding similar femoral arterial drug concentration at rest vs. exercise. For PE, drug concentrations of 0.025, 0.05 and 0.1 (rest) vs. 0.4, 0.8 and 1.6 μg/kg/min (exercise) were used, which resulted in theoretical drug deliveries of 0.12, 0.29 and 0.70 vs. 0.13, 0.29

and 0.60 μg/kg/l, respectively, p=ns. Similarly for BHT, 2.5 and 5 (rest) vs. 40 and 80 μg/kg/min (exercise) gave deliveries of 12 and 32 vs. 15 and 29 μg/kg/l, respectively, p=ns. When comparing the responses to these flow-adjusted doses, the PE-induced changes in FAD were significantly smaller during exercise compared to rest for all doses (fig 6a). However, only the FBF response to the highest flow-adjusted dose of PE was statistically reduced during exercise, and FVC responses at rest and during exercise were not statistically different for PE (fig 6a). In contrast, the FBF and FVC responses to both flow-adjusted doses of BHT were significantly reduced during exercise (fig 6b).

Responses of PE and BHT at rest and during ramped exercise (protocol 4).

The responses of a single, flow-adjusted dose of the α-agonists PE (0.3 μg/kg/l) and BHT (15 μg/kg/l) during rest and ramped exercise from 7W-37W are presented in figure 7a and 7b, respectively. At rest, FAD changed significantly during both PE (9.4±0.3 to 8.2±0.5 mm, $p<0.01$) and BHT (9.5±0.3 to 8.9±0.3 mm, $p<0.01$). The change in FAD was significantly greater with PE compared to BHT ($p<0.05$). PE and BHT produced comparable reductions in FBF (-44±7 vs. -50±5%, PE vs. BHT) and FVC (-44±7 vs. -50±5%, PE vs. BHT).

Ramped exercise at 7-37W significantly increased HR (57±4 at rest to 65±2, 72±3, 77±2 and 82±2 beats/min during 7, 17, 27, and 37W) with no significant change in MAP. FBF increased during exercise to approximately 1250, 2000, 2750 and 3400 ml/min (7, 17, 27 and 37W, respectively) with no significant differences between the two bouts of exercise.

PE significantly reduced FAD at 7W and 17W, decreased in FBF at 7W and 17W, and reduced FVC at 7W, 17W, and 27W (fig 7a). At the highest workload (37W), the effects of PE were abolished. The doses of PE used in this protocol caused no significant changes in MAP or HR. In contrast, BHT caused no significant differences in FAD, FBF or FVC at any intensity (fig 7b). BHT caused small (3-4 mmHg) but statistically significant decreases in MAP at 17W and 27W without changing HR. While the relative changes in FBF and FVC were significantly attenuated at all levels of exercise compared to rest for both agonist drugs, the differences between PE and BHT reached statistical significance only at the 7W intensity (fig 8).

In a subset of subjects (n=5), the effects of an additional double-dosing of PE and BHT were studied during 7W exercise. For all parameters, this doubling of doses caused no significant changes in the responses to the drugs, indicating that the effects seen with the doses chosen were already near maximal.

Comparison of ultrasound Doppler and thermodilution for measuring femoral blood flow during exercise.

Femoral blood flows measured during exercise with ultrasound Doppler (FBF_D) and thermodilution (FBF_{TD}) were always performed within the same minute of steady state exercise. The average FBF_{TD} and FBF_D during 27W exercise before drug infusions were 2960±240 ml/min and 2890±400 ml/min (mean±95% confidence intervals) (p=ns). The PE and BHT-induced changes were small during exercise with both methods, and at no measurement point did the two methods yield significantly different results ($p \geq 0.29$ for

all paired *t*-tests before Bonferroni correction). The difference in FVC between PE and BHT did not reach statistical significance when using FBF_D as the basis for calculating FVC, but the PE-effect remained significant. The two methods also yielded very similar results during the ramped exercise protocol, which at no measurement point were statistically different ($p \geq 0.11$ for all paired *t*-tests before Bonferroni correction). The individual and summary data for simultaneous measurements of FBF using Doppler and thermodilution during the first bout of ramped exercise at 7, 17, 27, and 37W are compared in figure 9. As in protocol 3, FBF_{TD} showed less variability within the group than FBF_D with the exception of 37W, where the variability was similar. The PE and BHT-induced changes in blood flow were not different when comparing the two methods, and the PE-induced decreases in FBF_D and FVC during 7 and 17W remained significant. The difference between PE and BHT-responses for FBF_D during 7W did not reach statistical significance.

DISCUSSION

There are several major new findings from the present study. First, in dose-response studies α_1- and α_2-adrenoreceptor agonists produce comparable maximal vasoconstriction in the resting leg. Second, the small α_2-mediated compared to the large α_1-mediated decrease in femoral artery diameter provides functional evidence that α_2-receptors are located predominantly distal in the vascular tree. Third, we demonstrate for the first time metabolic inhibition of both α_1- and α_2-mediated vasoconstriction in the human leg. Finally, we show that α_2-mediated vasoconstriction is more sensitive to metabolic inhibition in the exercising human thigh, since α_2 is completely inhibited even at low workloads, whereas α_1 becomes progressively inhibited with increasing workloads. Collectively, these findings provide novel insight regarding the contribution of α-adrenoreceptor subtypes to the control of muscle blood flow at rest and during exercise.

Evidence for heterogeneous distribution of α -adrenoreceptors.

Our finding that femoral artery diameter (FAD) was reduced to a much larger extent following α_1-compared to α_2-agonist administration provides functional evidence of a heterogeneous distribution of α-adrenoreceptors in the skeletal muscle vasculature of the human leg. This extends observations in the rat (Anderson & Faber, 1991) demonstrating a predominance of α_1-adrenoreceptors in the upstream, conduit arteries and α_2-receptors in the resistance arterioles of the microcirculation. Thus, the present study has advanced

the concept first identified in animals (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; McGillivray-Anderson & Faber, 1991) that large upstream arteries contain both α_1- and α_2-receptors for the control of arterial blood pressure, while the nutrient arterioles contain predominantly α_2-receptors for the fine control of tissue perfusion.

α_1- vs. α_2-mediated vasoconstriction at rest.

The maximal reduction in resting FBF following α_2-agonist (BHT) application was similar to that seen following α_1-agonist (PE) administration. This extends similar observations in the dog hind limb following intra-arterial agonist infusion of PE (selective for α_1) and clonidine (selective for α_2) at rest (Buckwalter & Clifford, 2001). Conversely, local administration of selective α-antagonist agents in the resting human forearm indicated an apparent dominance of α_2-adrenoreceptor-mediated vascular tone (63% of basal tone) over α_1 (Dinenno *et al.*, 2002b). However, calculated differences in control of resting flow do not exclude similar maximal responses to α_1- vs. α_2-agonists. Furthermore, dissimilar α_1-responsiveness has recently been reported in the human arm and leg (Jacob *et al.*, 2000; Pawelczyk & Levine, 2002), suggesting that these two limbs exhibit differences in α_1-adrenoreceptor sensitivity and distribution.

Metabolic inhibition of local vasoconstriction in exercising muscle.

To our knowledge, we have provided the first pharmacologic evidence of metabolic attenuation of α-adrenergic vasoconstriction during dynamic exercise in the human thigh. These findings extend results from studies in the human forearm (Tschakovsky *et al.*, 2002). However, we believe there are several major advantages of using knee-extensor exercise over handgrip in this context. First, direct and accurate measurements of blood flow during exercise are feasible by the thermodilution and ultrasound Doppler methods (Andersen & Saltin, 1985; Walloe & Wesche, 1988; Radegran & Saltin, 1998). Second, the fraction of blood flow in the femoral artery reaching the thigh muscles during knee-extensor exercise is more than 95% while using an occlusive cuff below the knee (Savard *et al.*, 1988). In contrast, rhythmic handgrip cannot be sustained with an occlusive wrist cuff (Tschakovsky *et al.*, 2002), and the fraction of brachial artery blood flow reaching contracting muscle at low intensity exercise is below 90% (Wahren, 1966). Third, knee-extensor exercise up to 30W (around 40% of maximum) can be sustained for several hours with steady state blood flow and pressure, without fatiguing, and with no significant activation of the sympathetic nervous system as measured by plasma norepinephrinee (Turcotte *et al.*, 1992; Steensberg *et al.*, 2002). In contrast, to sustain rhythmic handgrip for more than 10 minutes requires very low intensities (10% of maximum) (Wahren, 1966; Rosenmeier *et al.*, 2003). At higher intensities fatigue and evidence of increasing activation of muscle sympathetic nerve activity quickly become apparent (Tschakovsky *et al.*, 2002). In the present study, these unique attributes of the human knee-extensor model of exercise allowed both definition of the level of intensity

required for metabolic inhibition of sympathetic vasoconstriction and definition of the pharmacological dose-responses at a given exercise intensity.

Previous studies in humans and animals have pointed to a post-junctional site of interaction between skeletal muscle metabolic events and α-adrenergic vasoconstriction. In this regard, responses to reflex-mediated increases in muscle sympathetic nerve activity, direct sympatho-neural stimulation, indirect acting sympathomimetics (tyramine) and intra-arterial directly acting α-agonists are all attenuated during exercise (Ruble *et al.*, 2002; Tschakovsky *et al.*, 2002; Keller *et al.*, 2003; Rosenmeier *et al.*, 2003). A wide array of factors contributing to the observed alteration in vascular responsiveness during exercise have been proposed, including vasoactive substances such as adenosine (Laughlin *et al.*, 1989; Saltin *et al.*, 1998; Radegran & Hellsten, 2000), prostaglandins and thromboxanes (Karamouzis *et al.*, 2001), the indirect effect of increased muscle temperature (Cooke *et al.*, 1984), hypoxia (Hansen *et al.*, 2000a), and acidosis (McGillivray-Anderson & Faber, 1990). These factors are not mutually exclusive. It is noteworthy that several different protocols performed in both rodents and humans have identified skeletal muscle production of nitric oxide as an important metabolic event. Lack of skeletal muscle nitric oxide synthase or pharmacological blockade of nitric oxide production has been accompanied by loss of metabolic inhibition of sympathetic vasoconstriction (Thomas & Victor, 1998; Sander *et al.*, 2000; Chavoshan *et al.*, 2002; Fadel *et al.*, 2003; Thomas *et al.*, 2003). However, recent evidence suggests no significant differences in the effects of α-agonists infused intra-arterially in the human forearm during handgrip exercise before and after pharmacological blockade of nitric

oxide synthesis (Dinenno & Joyner, 2003). This study clearly demonstrates exercise-induced inhibition of sympathetic vasoconstriction during handgrip and therefore suggests that nitric oxide may not be the only important signaling molecule mediating metabolic inhibition of sympathetic vasoconstriction.

Stimulation of the α-receptors in the exercising limb has often produced some degree of vasoconstriction. This was also the case in the present study for phenylephrine during lower exercise intensities. Conflicting evidence exists regarding the importance of functional sympatholysis versus sympathetic vasoconstriction in exercising skeletal muscle, and has been the subject of several comprehensive reviews (Laughlin, 1996; Rowell, 1997; Delp & Laughlin, 1998; Buckwalter & Clifford, 2001). The principal point of contention centers on the paradox of vasoconstriction of the exercising muscle vasculature, which seems counterproductive to the effort of increasing blood flow to meet the metabolic demands of the muscle. Several studies have addressed whether blood flow to exercising muscle or blood pressure is prioritized during heavy exercise of large muscle groups, with conflicting results (Secher *et al.*, 1977; Savard *et al.*, 1989; Richter *et al.*, 1992; Strange, 1999). However, it is now well accepted that perfusion of the contracting skeletal muscle is ultimately a balance between metabolic vasodilation and sympathetic vasoconstriction (Joyner & Thomas, 2003). The underlying mechanisms guarding this balance are still incompletely understood.

α_1- vs. α_2-mediated vasoconstriction during exercise.

Experimental evidence in microvascular and animal preparations have suggested that α_2-mediated vasoconstriction is more sensitive to metabolic inhibition than α_1-mediated vasoconstriction (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Buckwalter *et al.*, 2001; Ruble *et al.*, 2002; Joyner & Thomas, 2003). Thus, the vasoconstrictor responses to specific α_2-agonists such as UK 14304 and clonidine were more effectively inhibited during exercise than α_1-agonists such as phenylephrine and the non-specific agonist norepinephrine. Microvascular studies in rats have identified a heterogenous distribution of α-adrenoreceptors, with α_2-adrenoreceptors located predominantly in the peripheral vascular bed (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991). Taken together, these findings have prompted the hypothesis that preserved α_1-responsiveness would help to maintain blood pressure by keeping vascular tone in arteries and large arterioles, while attenuation of α_2-responsiveness in smaller arterioles would help increase nutrient blood flow to the exercising muscle fibers.

In humans, mild handgrip exercise was recently shown to attenuate both phenylephrine and clonidine-mediated vasoconstriction compared to rest (Rosenmeier *et al.*, 2003). However, this study used only one level of exercise and one dose (flow-adjusted) of each drug, and therefore was not powered to detect differences between the attenuation of α_1- vs. α_2-mediated vasoconstriction. The present study extends these findings to the human leg, because we also found evidence for attenuation of both α_1- and α_2-mediated vasoconstriction, and our dose-response curves provide evidence that

even the maximal responses are attenuated. Using the ramped exercise protocol, we also demonstrated a residual vasoconstriction to phenylephrine (α_1-agonist) at low and moderate exercise intensity, whereas BHT-933 (α_2-agonist) did not produce vasoconstriction at any exercise intensity. Thus, a major contribution of the present study is the demonstration of a greater sensitivity of α_2- than α_1-adrenoreceptor subtypes to the metabolic byproducts produced during exercise in the human thigh.

Degree of exercise-induced inhibition of α-adrenergic responses.

Previous studies and the present study have demonstrated seemingly different degrees of exercise-induced attenuation of α-adrenergic vasoconstriction. For non-specific α-adrenergic or α_1-adrenergic agonists some previous animal studies have shown very little exercise-induced attenuation of the response (Thomas *et al.*, 1994; Buckwalter *et al.*, 1998b). In contrast, more recent animal studies conclude that phenylephrine-responses are attenuated during exercise (Buckwalter *et al.*, 2001). Furthermore, recent human forearm studies (Dinenno & Joyner, 2003; Rosenmeier *et al.*, 2003) and the present study agree that even mild exercise causes attenuation of the phenylephrine-response such that maximal responses seen during exercise are around 10-20% of blood flow, compared to 50-60% at rest. The reason for this apparent development of results from earlier to more recent studies is not clear. For α_2-adrenergic agonists, animal studies do differ with regards to degree of exercise-induced attenuation of the response. In rat hind limb, heavy evoked exercise caused a complete inhibition of the response to the α_2-agonist UK14304 (Thomas *et al.*, 1994), whereas in the conscious dog, the hind limb response to the α_2-

agonist clonidine remained significant, around 15% at heavy running (Buckwalter & Clifford, 2001). The differences in these studies were several, including species and choice of α_2-agonist. Regarding the latter, in vitro studies have identified UK14304 as a full agonist but clonidine as a partial agonist (Wise *et al.*, 1997). In the recent human handgrip studies, the response to clonidine also remains at around 15% of brachial blood flow during mild exercise (Dinenno *et al.*, 2003; Rosenmeier *et al.*, 2003), whereas in the present study there was no detectable response in the thigh circulation to BHT-933 even during mild exercise. The obvious differences between these studies include the choice of limb and α_2-agonist. The choice of limb may explain part of the difference, because non-exercising muscle and tissue like the skin receive a larger proportion of brachial blood flow during handgrip than femoral blood flow during knee-extensor exercise. The choice of α_2-agonist is also likely to be important, because clonidine has been reported less specific compared to BHT-933 (Jie *et al.*, 1984). Thus, the higher flow-adjusted doses of clonidine used during exercise in other studies may cause α_1-adrenergic receptor stimulation.

Significance of Functional Sympatholysis in the leg.

The leg represents a large muscle mass with an enormous capacity to vasodilate during exercise, reaching up to 2.5 liters/kg/min (Andersen & Saltin, 1985) and often receiving the majority of cardiac output (Rowell, 1993). Others have observed that the increase in skeletal muscle blood flow accompanied by the increase in fraction of cardiac output with exercise means that small changes in vascular conductance can greatly influence arterial

pressure (O'Leary, 1991; Tschakovsky *et al.*, 2002). Thus, exercise produces a condition that demands a precise balance between neural and metabolic control of flow to maintain homeostasis. The vascular bed of the leg has been described as a "sleeping giant" that may necessitate a hemodynamic response unique to this large muscle group (Andersen & Saltin, 1985). Accordingly, the observed attenuation of α-adrenergic vasoconstriction may be a protective mechanism, insuring adequate blood flow to the exercising tissue in the face of increased sympathetic outflow.

As noted above, muscle sympathetic nervous activity, measured by plasma norepinephrine, does not increase significantly at knee-extensor exercise intensities below 30W (Turcotte *et al.*, 1992; Steensberg *et al.*, 2002). Since metabolic attenuation of α-adrenergic vasoconstriction is evident in the present study even at intensities well below 30W, it would seem sympathetic neural activation does not occur until well after the thigh muscle is "protected" by functional sympatholysis. This extends similar findings in studies of handgrip exercise (Hansen *et al.*, 1996).

Experimental Limitations.

The potential experimental limitations of the present study are related to the use of exogenous vasoconstrictors administered luminally. Physiological control of peripheral adrenergic receptors takes place at the abluminal side, where norepinephrine released from sympathetic boutons primarily act on proximate receptors located on vascular smooth muscle cells. Sympathetic boutons are more abundant at the level of the resistance arterioles compared to large arteries. When agonists are administered intra-

arterially both luminal endothelial receptors and abluminal smooth muscle receptors are activated. However, in previous studies in the human forearm the responses to direct acting adrenergic agonists and the indirect sympathomimetic tyramine are attenuated to similar degrees during handgrip (Tschakovsky *et al.*, 2002; Rosenmeier *et al.*, 2003).

In these human studies, as well as in some animal studies, the sympathomimetic dosing has been "flow-adjusted" to achieve similar intra-arterial drug-concentration (Buckwalter *et al.*, 1998b; Ruble *et al.*, 2002; Tschakovsky *et al.*, 2002; Rosenmeier *et al.*, 2003). Furthermore, in several studies α-adrenergic responses during exercise have been compared with the responses seen during local pharmacological vasodilation administered to match exercise hyperemia (Tschakovsky *et al.*, 2002; Rosenmeier *et al.*, 2003). When phenylephrine, clonidine and tyramine are superimposed on adenosine or nitroprusside-induced vasodilation, the result has invariably been large decreases in blood flow. This approach has served to validate that the diminished vasoconstrictor responses during exercise are not related to changes in drug delivery during increases in blood flow. It should be noted that flow-adjusting the dose of sympathomimetic does not secure adjustment of drug-concentration in the vascular wall interstitium or receptor occupancy. The higher absolute drug dose would tend to lower transit time and decrease receptor occupancy when using flow-adjusted dosing during exercise. This may be a concern, especially when the drugs are administered as bolus-injections (Buckwalter *et al.*, 1998a; Ruble *et al.*, 2002; Tschakovsky *et al.*, 2002). Another caveat to flow-adjusted doses is that the resultant intra-arterial α-agonist concentration will be different during the latter part of drug administration in a condition where flow does not change much in response

to the agonist drug (i.e. exercise) compared to a condition where flow decreases by about 50% in response to the agonist drug (i.e. resting pharmacologic vasodilation) (Rosenmeier *et al.*, 2003). In the present study, the wide dose-response ranges in combination with flow-adjusted dosing (both by design and post-hoc) have minimized these potential concerns of using intra-arterial infusion of agonists.

When using pharmacological tools, the specificity of the drugs should always be considered. Phenylephrine has the ability to activate β-adrenergic receptors (Torp *et al.*, 2001). This effect is unlikely to be of major importance in the present study, since exercise attenuated phenylephrine-responses to similar degrees both with and without complete beta-blockade by the non-specific β-blocker propranolol (protocols 3 and 4). We chose phenylephrine, because it is well accepted to be a relatively specific α_1-agonist. We chose BHT-933 as an α_2-agonist rather than the classic drug clonidine, because in a previous study the actions of clonidine were inhibited by both the α_1- (prazosin) and α_2- (yohimbine) antagonists, whereas BHT-933 actions are only inhibited by yohimbine (Jie *et al.*, 1984). Our data (protocol 2) supported the specificity of PE for α_1-receptors since the α_2-antagonist yohimbine caused no decrease in the response to PE, and provided the first evidence in humans that BHT actions in the human thigh are largely inhibited by an α_2-antagonist.

One disadvantage of using the knee-extensor exercise model is that the maximal drug-doses during exercise may have minor systemic effects. The highest intra-femoral doses of PE caused significant changes in blood pressure and heart rate during exercise, but not during rest. The explanation for this differential effect is likely related to the

different transit time of PE during the two conditions. At rest the mean transit time during PE-administration in the thigh is likely to be more than 15 seconds in the leg alone, while during exercise the transit time is decreased to 5 seconds (Bangsbo *et al.*, 2000). Thus, the uptake and degradation of PE may be incomplete during exercise. It is worth emphasizing that such a "flow through phenomenon" is unlikely to explain the exercise-induced inhibition of α-agonist effects, because simply increasing flow and lowering transit time by vasodilators in other studies have not been sufficient to inhibit α-agonist vasoconstriction (Dinenno & Joyner, 2003; Rosenmeier *et al.*, 2003).

For the present study, the dose-response and ramped exercise protocols both included the responses to phenylephrine 0.3μg/kg/l and to BHT-933 15μg/kg/l at rest and during 27W knee-extensor exercise. In both protocols, the overall findings were similar, because phenylephrine and BHT-933 caused large decreases in femoral blood flow and conductance at rest, and visibly smaller responses during exercise. It should be noted, however, that the blood flow decrease in response to phenylephrine during exercise reached significance only in the dose-response protocol, whereas the response to phenylephrine during exercise vs. rest was only significant in the ramped exercise protocol. The reason for this difference in the responses to phenylephrine in the two protocols is unclear, but could be related to the difference in the duration and total dose of the phenylephrine-infusion. The responses to BHT-933 during 27W exercise were very similar in both protocols.

Conclusions and Potential Clinical Significance.

We have identified a *functionally differential* distribution of α-adrenoreceptor subtypes in the vasculature of the human leg. Furthermore, we have demonstrated a higher sensitivity of α_2-mediated vasoconstriction compared to α_1-mediated vasoconstriction to the metabolic events taking place during mild thigh muscle exercise. Strikingly, we have observed that moderate intensity knee-extensor exercise completely inhibits the vasoconstrictor actions of intra-arterially administered exogenous sympathomimetics. Knowledge of these mechanisms may provide the clinician with a better understanding of the functional consequences of pathological vascular disorders associated with age, diabetes, heart failure, and hypertension, and present possible diagnostic techniques and methods of treatment. For example, an age-related reduction in α-adrenergic responsiveness specific to α_1-receptors has recently been described (Dinenno *et al.*, 2002a). This emphasizes the need for a better understanding of subtype contributions to peripheral vascular control. Furthermore, it has been demonstrated that patients suffering from congestive heart failure experience diminished skeletal muscle perfusion at rest and during sub-maximal and maximal exercise, with accompanying increased vascular resistance (Sullivan *et al.*, 1989). Recent experimental and clinical trials indicate that this relative hypo-perfusion is related to an inability of the skeletal muscle to adequately vasodilate in opposition to the increased sympathetic nerve activity that accompanies exercise (Johnson *et al.*, 1999; Notarius *et al.*, 1999). Since these patients might benefit from exercise as prophylaxis and as part of a cardiac rehabilitation program (Monchamp

& Frishman, 2002), knowledge of α-adrenoceptor contribution to the control of blood flow might provide a therapeutic target in this population.

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FIGURE LEGEND

FIGURE 1: Experimental protocols 1-4 represented graphically in 4 panels. Time-courses are given in minutes on the top axis of each panel. **Protocol 1:** the β-receptor agonist isoproterenol (1, 2, 4, and 8 ng/kg/min, 2-min infusions) was administered before and after 150 minutes of β-blockade with propranalol (12.5 μg/kg bolus and 0.125 μg/kg/min maintenance). **Protocol 2:** the selective α_2-adrenoreceptor agonist BHT-933 (BHT, 20 and 40 μg/kg/min, 2-min infusions) and the selective α_1-agonist phenylephrine (PE, 0.4 and 0.8 μg/kg/min, 2-min infusions) were applied before and during the last 10 minutes of a 20 minute infusion of the α_2-antagonist yohimbine (5 μg/kg/min). **Protocol 3:** Dose-response for BHT (2.5-80 μg/kg/min, 2-min infusions) and PE (0.025-1.6 μg/kg/min, 2-min infusions) at rest and during 27W knee extensor exercise. **Protocol 4:** Flow-adjusted administration of BHT (15 μg/kg/L/min^2, 3-min infusions) and PE (0.3 μg/kg/L/min^2, 3-min infusions) at rest and during ramped exercise.

FIGURE 2: Femoral blood flow (FBF) during infusion of the non-selective β-agonist isoproterenol (1-8 ng/kg/min) before (black bars), and 150 min after initiation of non-selective β-blockade with propranolol (grey bars). Propranolol provided virtually complete blockade of isoproterenol-mediated vasodilation (n=9). *, $p<0.05$ compared to baseline and compared to responses after propranolol.

FIGURE 3: Specificity of BHT for α_2-adrenoreceptors was validated by measurements of femoral blood flow before and after selective α_2-blockade with yohimbine. **Top**

Panel: Administration of the selective α_2-agonist BHT (20 and 40 μg/kg/min) produced significant vasoconstriction (grey bars), and this effects was abolished following yohimbine (5 μg/kg/min) (hatched bars). **Bottom Panel:** The selective α_1-agonist phenylephrine (PE, 0.4 and 0.8 μg/kg/min) produced significant vasoconstriction both before (black bars) and after administration of yohimbine (n=7) (hatched bars). *, $p<0.05$ compared to baseline; δ , $p<0.05$ compared to BHT-responses after yohimbine.

FIGURE 4: Dose-response relationships for intra-arterial PE and BHT at rest. **Panel A:** During incremental doses of PE, femoral artery diameter (FAD, top) decreased 35%, with concomitant reductions in femoral blood flow (FBF, middle) and femoral vascular conductance (FVC, bottom). **Panel B:** During incremental doses of BHT, FAD decreased only slightly and only at the highest doses. However, FBF and FVC both decreased significantly, and of similar magnitudes to the responses seen during PE. *, $p<0.05$ compared to resting baseline, §, $p<0.05$ between comparable doses of PE and BHT.

FIGURE 5: Dose-response relationships for intra-arterial PE and BHT during 27W exercise. **Panel A:** During exercise, PE reduced FAD only at the highest dose (top). The changes in FBF (middle) and FVC (bottom) during PE were blunted compared to rest. A significant change in FVC occurred at the highest doses of PE compared to baseline and compared to the highest dose of BHT, and FBF also fell slightly during high doses of PE. **Panel B:** During exercise, the response to BHT was completely abolished, with no

significant change in FAD, FBF, or FVC, even at the highest dose. *, $p<0.05$ compared to exercising baseline, ¥, $p<0.05$ between comparable doses of PE and BHT.

FIGURE 6: Post-hoc flow-adjusted doses for PE and BHT at rest (triangles) and during 27W exercise (circles). **Panel A:** PE-induced reduction in FAD (top) was significantly smaller during exercise compared to rest for all flow-adjusted doses. However, only the FBF response (middle) to the highest flow-adjusted dose of PE was statistically reduced during exercise, and FVC responses (bottom) at rest and during exercise were not statistically different during flow-adjusted doses of PE. **Panel B:** BHT-induced reduction in FAD (top) was attenuated at the lower flow-adjusted dose, and the FBF (middle) and FVC (bottom) responses to both flow-adjusted doses of BHT were significantly reduced. ¥, $p<0.05$ compared to flow-adjusted dose-response at rest.

FIGURE 7: Absolute FBF and FVC during ramped exercise of 7-37W with superimposed administration of PE (A) and BHT (B). Both PE and BHT were flow-adjusted to the exercise-induced increase in FBF. **Panel A:** At lower workloads (7 and 17W), PE-induced decreases in FBF and FVC were seen (black bars), and this response was abolished at higher intensities. **Panel B:** BHT administration caused no changes in FBF or FVC (grey bars) at any exercise intensity. *, $p<0.05$ compared to pre-drug value for each level of exercise.

FIGURE 8: Changes in FBF (top) and FVC (bottom) during ramped exercise of 7-37W with superimposed administration of PE (black bars) and BHT (grey bars). All responses to BHT and PE during exercise were significantly smaller than at rest. At lower exercise intensities, PE produced significant changes in FBF and FVC, and these responses were significantly larger than the response to BHT at 7W. §, $p<0.05$ between PE and BHT at same level of exercise.

FIGURE 9: Comparison of Ultrasound Doppler and thermodilution techniques for determining femoral blood flow during knee-extensor exercise. The individual data shown by small solid black circles are obtained within the same minute for the two methods in 10 subjects during increasing levels of exercise intensity from 7-37W before administration of alpha-agonists. The mean values obtained by the two methods, shown in larger solid black circles, did not differ significantly at any intensity of exercise. The variation of the data obtained by the Doppler method were generally larger than the variation of the thermodilution data. Summary data are shown as mean ± 95% confidence intervals.

FIGURE 1

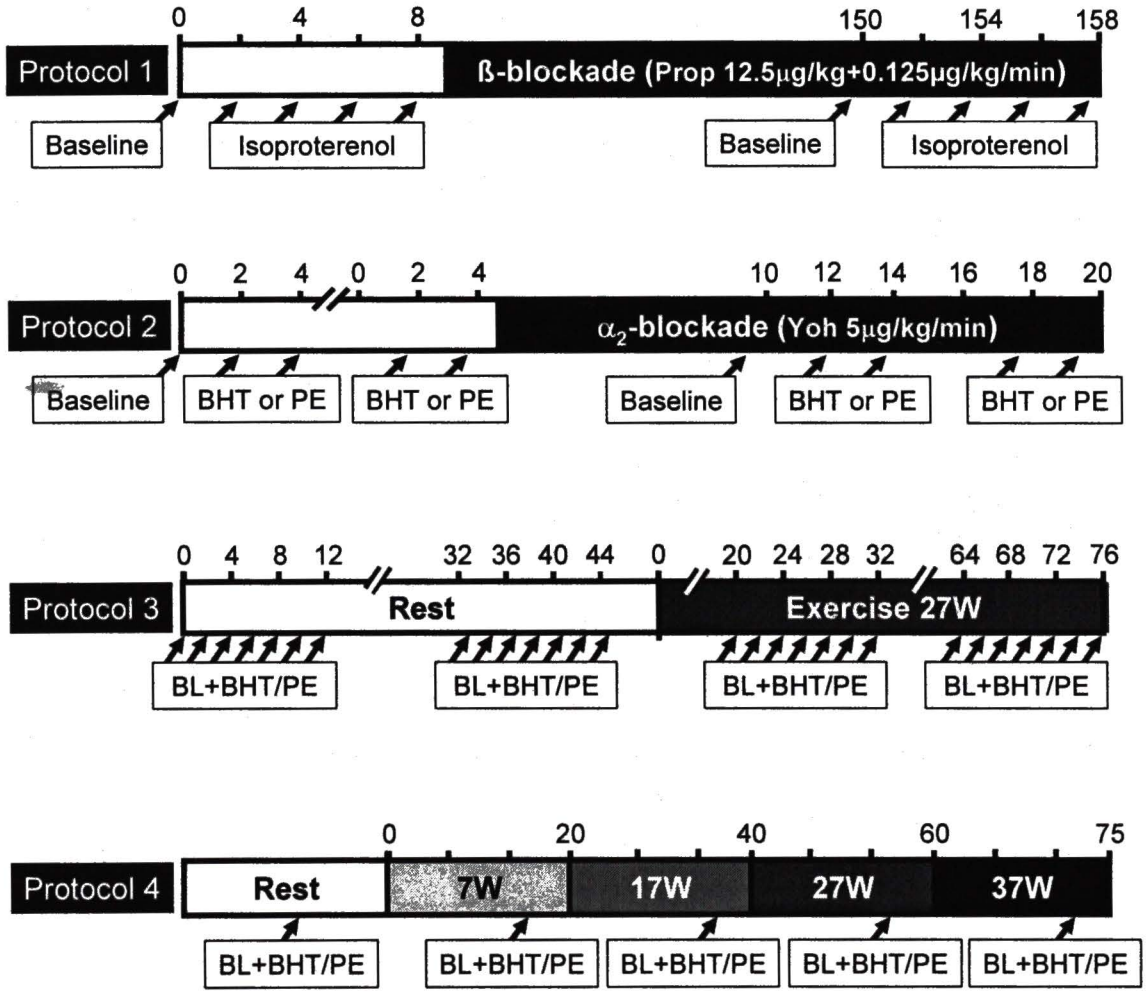

Protocol 1
0
4
8
150
154
158
ß-blockade (Prop 12.5µg/kg+0.125µg/kg/min)
Baseline
Isoproterenol
Baseline
Isoproterenol
Protocol 2
0
2
4
0
2
4
10
12
14
16
18
20
α2-blockade (Yoh 5µg/kg/min)
Baseline
BHT or PE
BHT or PE
Baseline
BHT or PE
BHT or PE
Protocol 3
0
4
8
12
32
36
40
44
0
20
24
28
32
64
68
72
76
Rest
Exercise 27W
BL+BHT/PE
BL+BHT/PE
BL+BHT/PE
BL+BHT/PE
Protocol 4
0
20
40
60
75
Rest
7W
17W
27W
37W
BL+BHT/PE
BL+BHT/PE
BL+BHT/PE
BL+BHT/PE
BL+BHT/PE

FIGURE 2

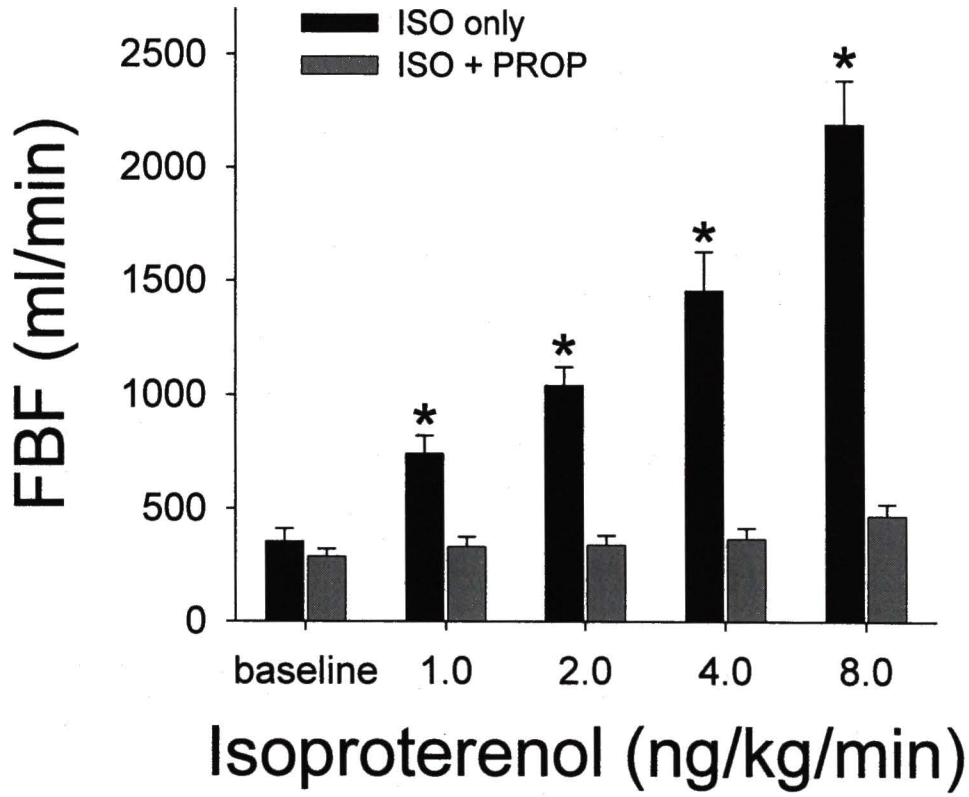
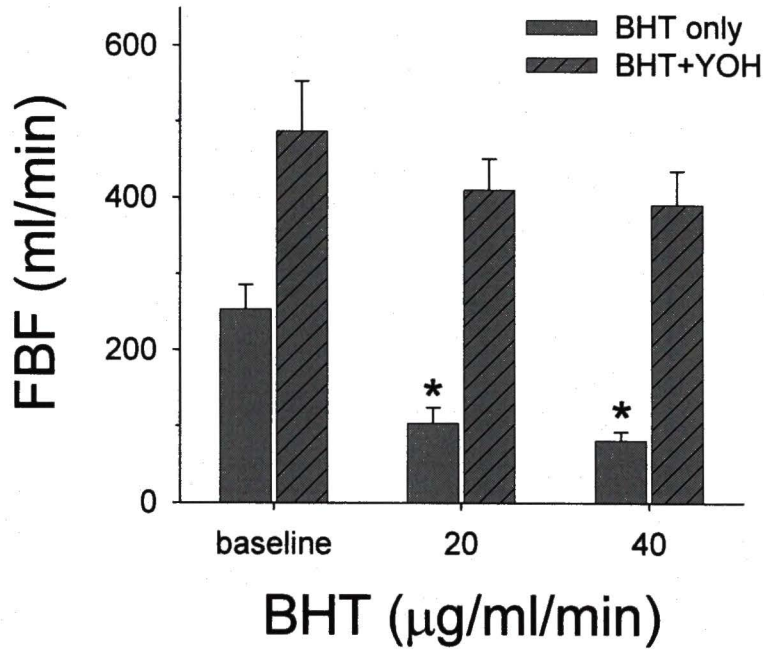

ISO only
ISO + PROP
FBF (ml/min)
2500
2000
1500
1000
500
0
*
*
*
*
baseline
1.0
2.0
4.0
8.0
Isoproterenol (ng/kg/min)

FIGURE 3


BHT only
BHT+YOH
600
400
200
0
FBF (ml/min)
*
*
baseline
20
40
BHT (μg/ml/min)

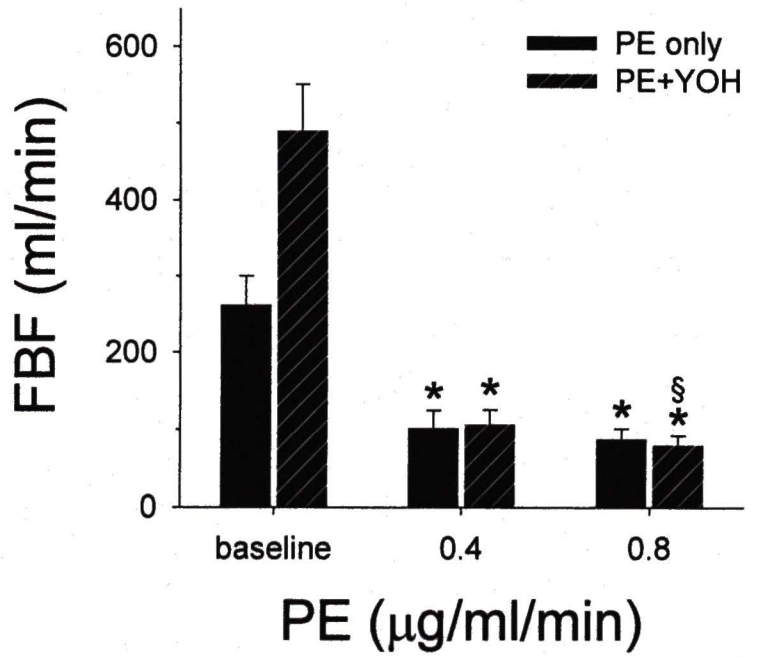

PE only
PE+YOH
600
400
200
0
FBF (ml/min)
*
*
*
§
*
baseline
0.4
0.8
PE (μg/ml/min)

FIGURE 4a

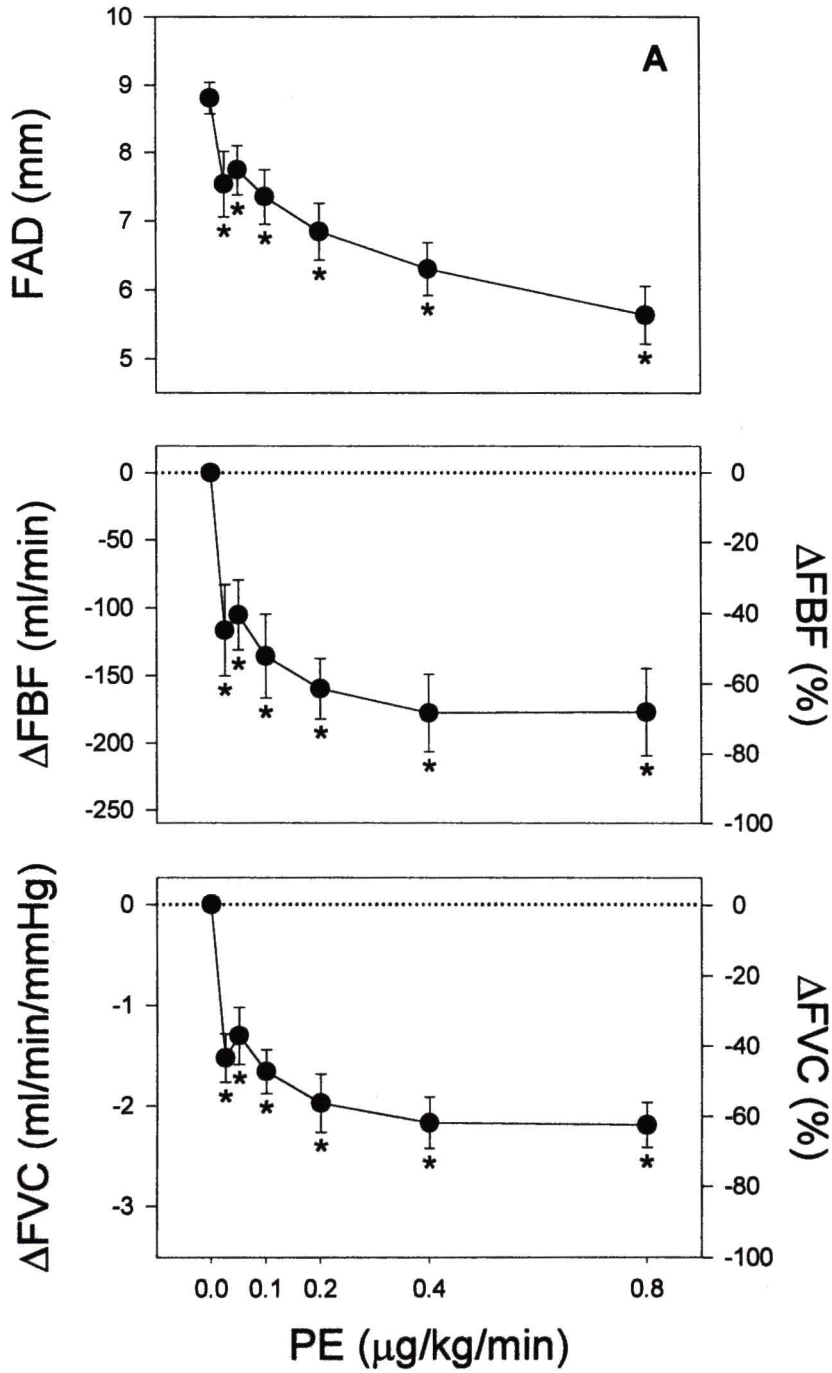

A
FAD (mm)
10
9
8
7
6
5
ΔFBF (ml/min)
0
-50
-100
-150
-200
-250
ΔFBF (%)
0
-20
-40
-60
-80
-100
ΔFVC (ml/min/mmHg)
0
-1
-2
-3
ΔFVC (%)
0
-20
-40
-60
-80
-100
0.0
0.1
0.2
0.4
0.8
PE (μg/kg/min)

Figure 4b

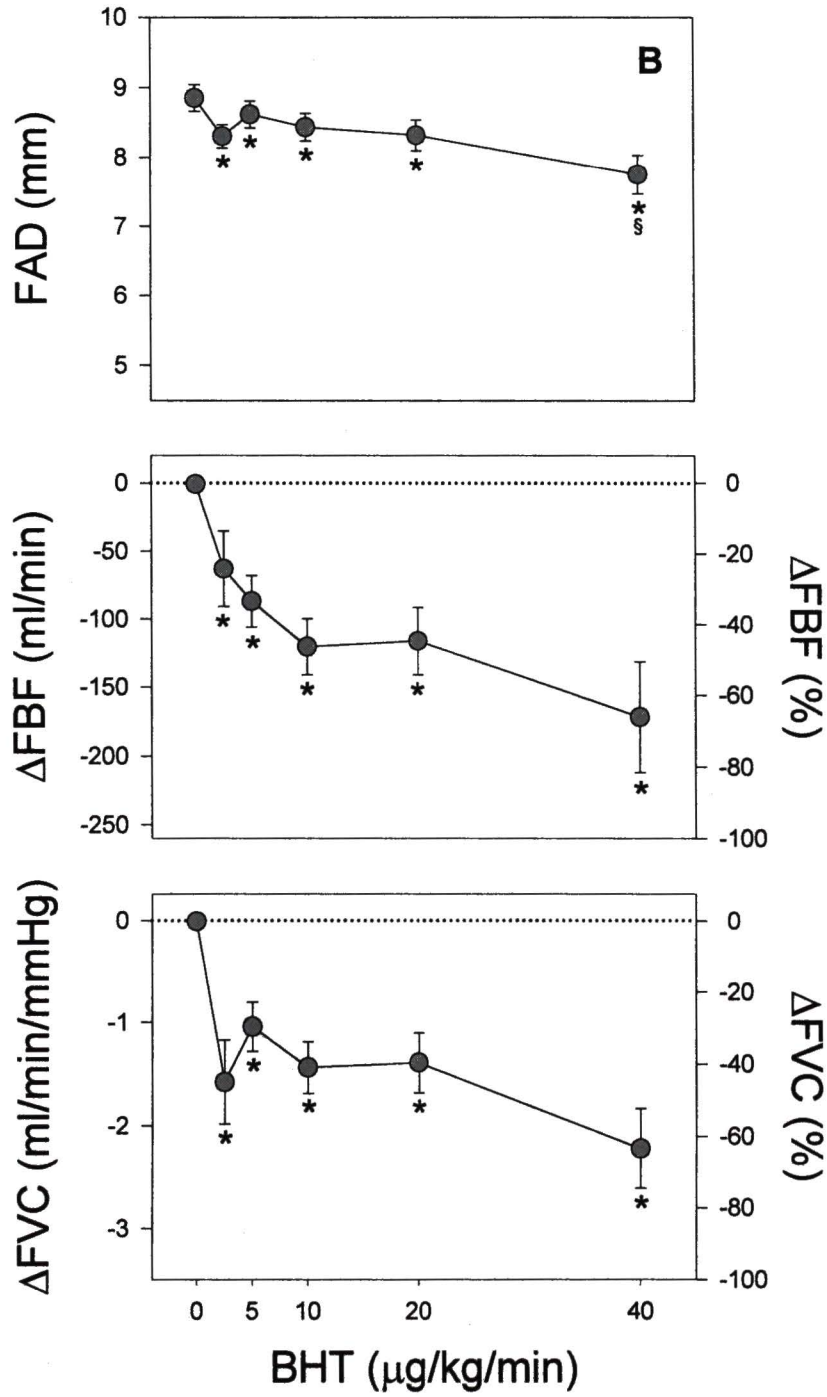

B
FAD (mm)
10
9
8
7
6
5
*
*
*
*
*
§
ΔFBF (ml/min)
0
-50
-100
-150
-200
-250
ΔFBF (%)
0
-20
-40
-60
-80
-100
ΔFVC (ml/min/mmHg)
0
-1
-2
-3
ΔFVC (%)
0
-20
-40
-60
-80
-100
0
5
10
20
40
BHT (μg/kg/min)

FIGURE 5a

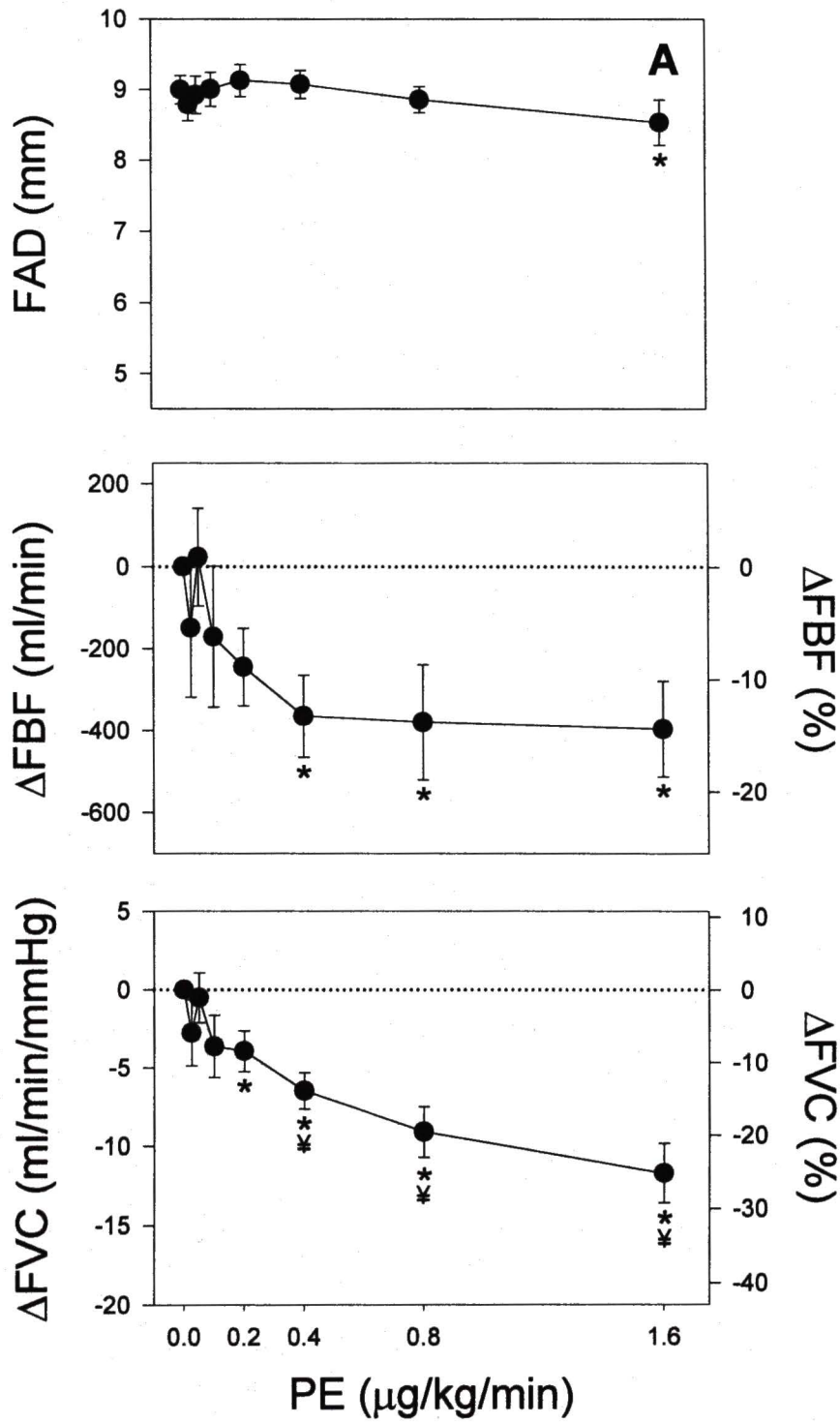

A
FAD (mm)
10
9
8
7
6
5
ΔFBF (ml/min)
200
0
-200
-400
-600
ΔFBF (%)
0
-10
-20
ΔFVC (ml/min/mmHg)
5
0
-5
-10
-15
-20
ΔFVC (%)
10
0
-10
-20
-30
-40
0.0
0.2
0.4
0.8
1.6
PE (μg/kg/min)

FIGURE 5b

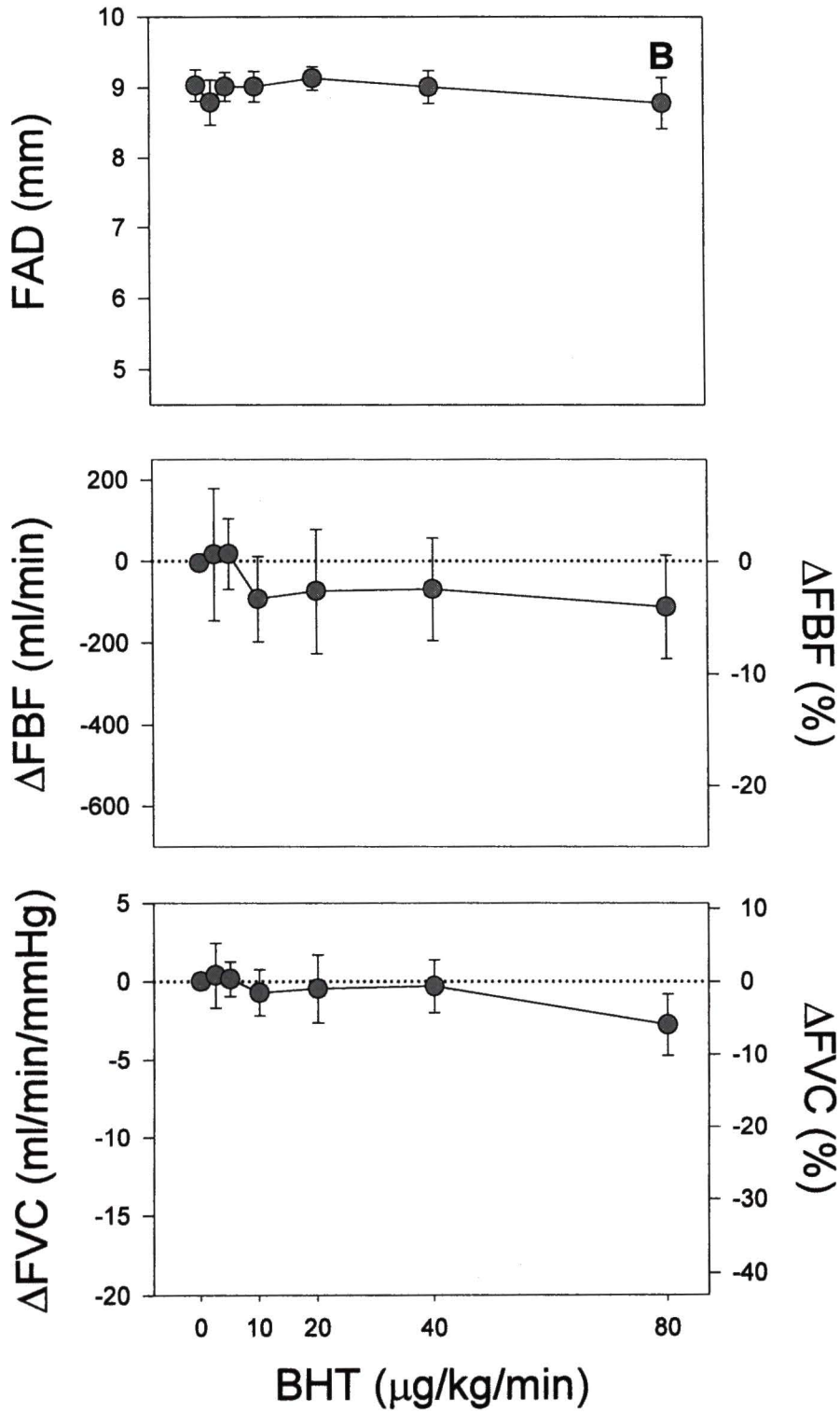

B
FAD (mm)
10
9
8
7
6
5
ΔFBF (ml/min)
200
0
-200
-400
-600
ΔFBF (%)
0
-10
-20
ΔFVC (ml/min/mmHg)
5
0
-5
-10
-15
-20
ΔFVC (%)
10
0
-10
-20
-30
-40
0
10
20
40
80
BHT (μg/kg/min)

FIGURE 6a

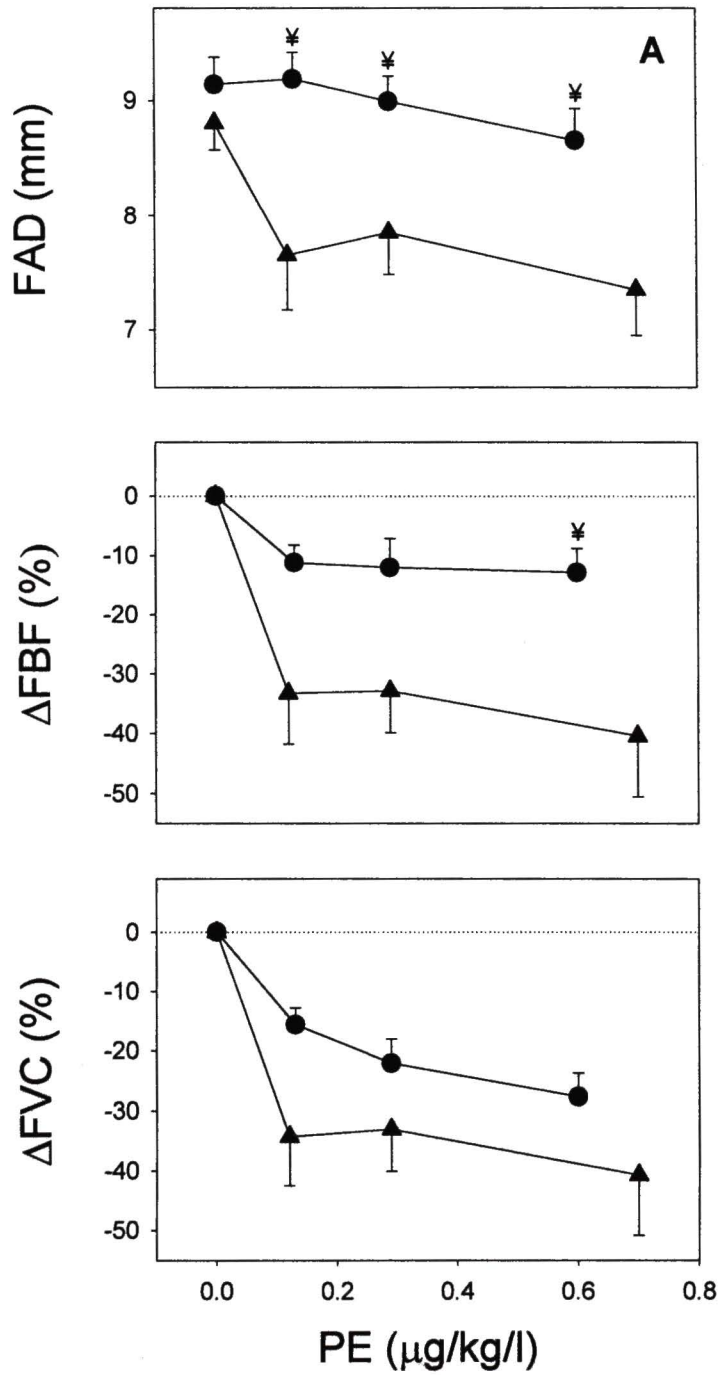

A
FAD (mm)
9
8
7
¥
¥
¥
ΔFBF (%)
0
-10
-20
-30
-40
-50
¥
ΔFVC (%)
0
-10
-20
-30
-40
-50
0.0
0.2
0.4
0.6
0.8
PE (μg/kg/l)

FIGURE 6b

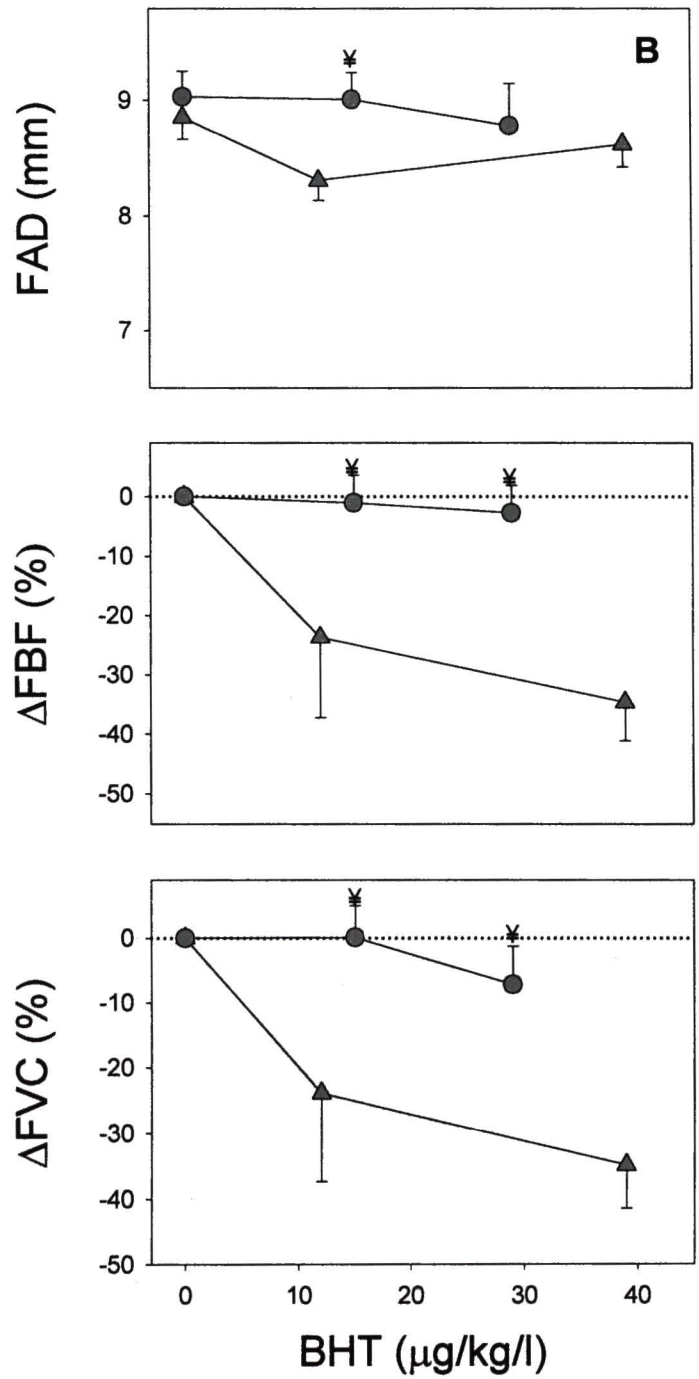

B
FAD (mm)
9
8
7
ΔFBF (%)
0
-10
-20
-30
-40
-50
ΔFVC (%)
0
-10
-20
-30
-40
-50
0
10
20
30
40
BHT (µg/kg/l)

FIGURE 7a

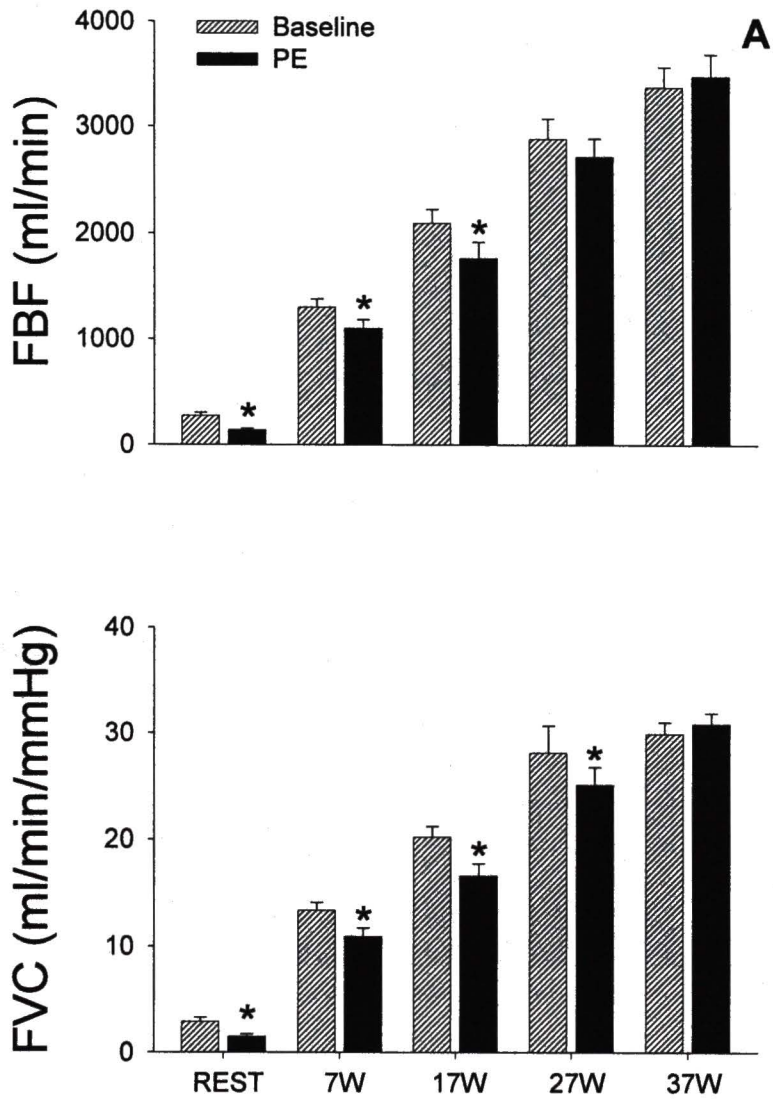

A
Baseline
PE
FBF (ml/min)
4000
3000
2000
1000
0
FVC (ml/min/mmHg)
40
30
20
10
0
REST
7W
17W
27W
37W

FIGURE 7b

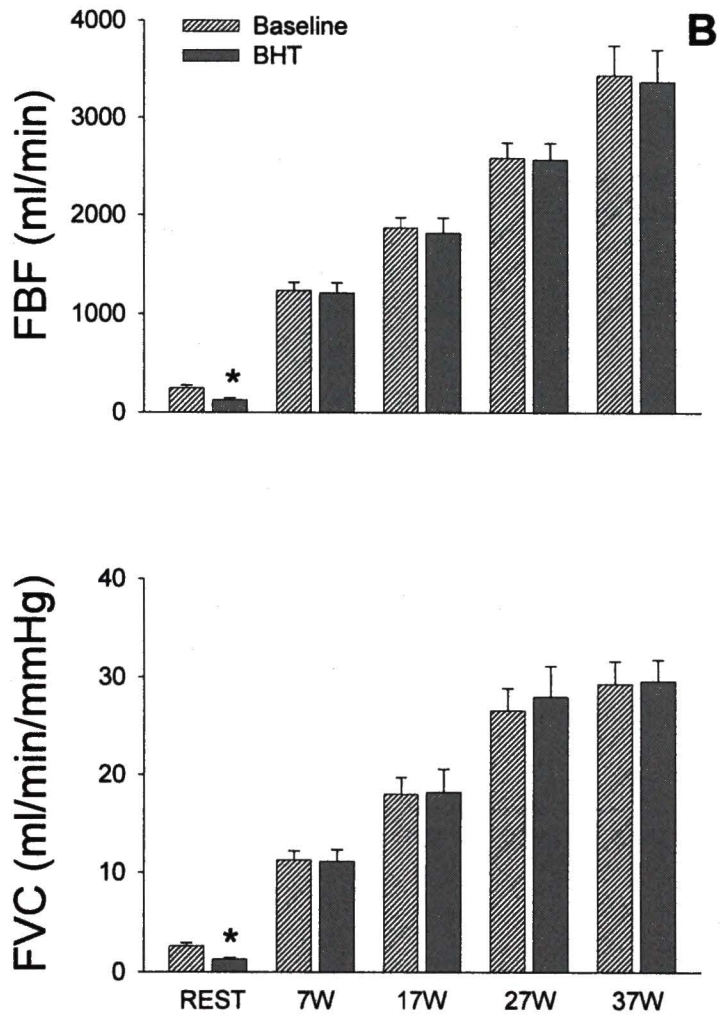

Baseline
BHT
B
FBF (ml/min)
4000
3000
2000
1000
0
*
FVC (ml/min/mmHg)
40
30
20
10
0
*
REST
7W
17W
27W
37W

FIGURE 8

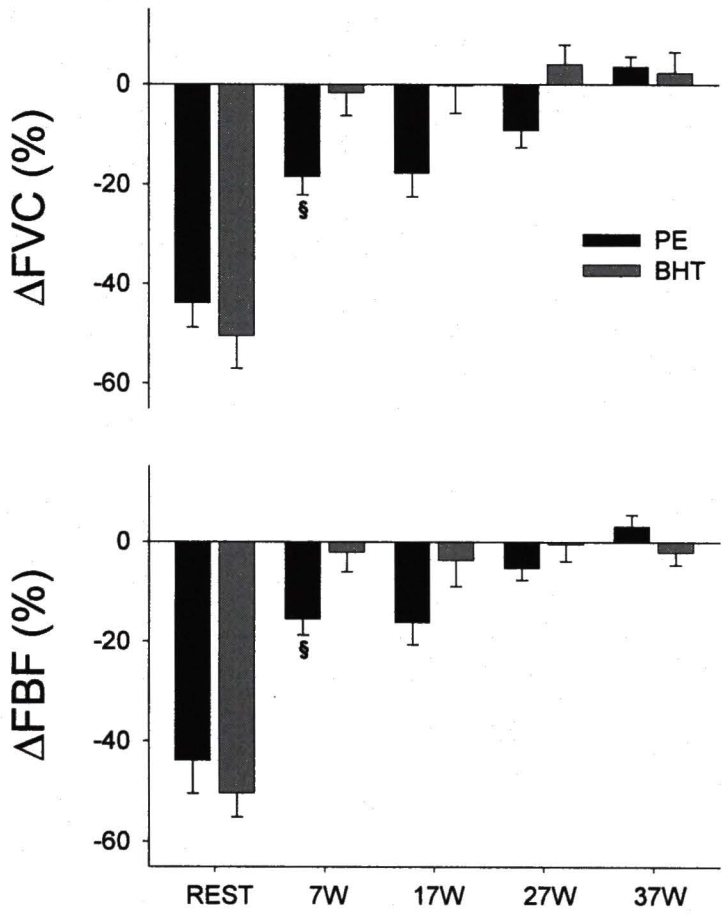
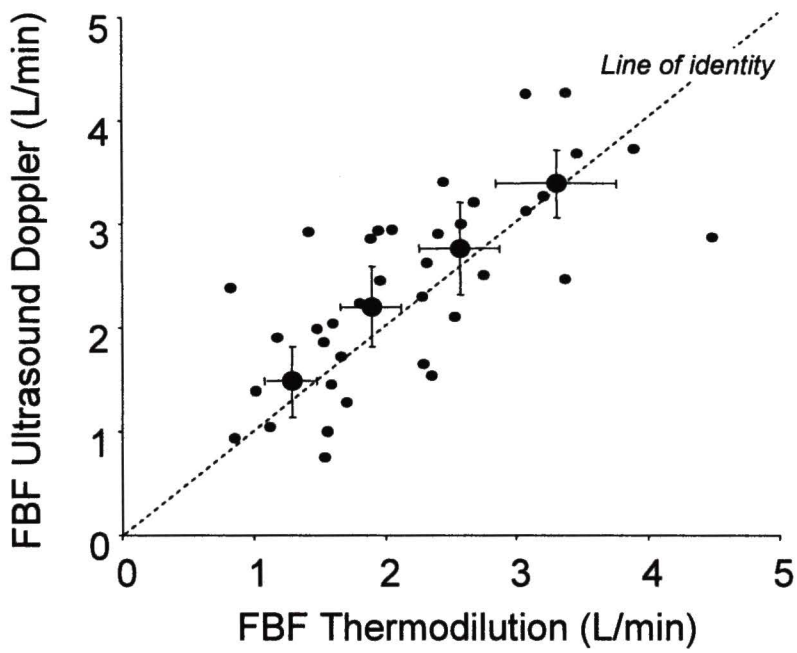


FIGURE 9


FBF Ultrasound Doppler (L/min)
FBF Thermodilution (L/min)
Line of identity
0
1
2
3
4
5

CHAPTER III

Dynamic Baroreflex Control of Peripheral Hemodynamics in Humans.

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Running head: Baroreflex entrainment of the peripheral circulation

Key Words: Spectral analysis; Sinusoidal neck pressure; Carotid Baroreflex; Arterial blood pressure

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ABSTRACT

While carotid baroreflex (CBR) control of heart rate (HR) and arterial blood pressure (ABP) have been well defined, reflex control of peripheral hemodynamics has not been studied extensively in humans. Furthermore, the dynamic properties of CBR function are not well understood. Therefore, we sought to use oscillatory CBR unloading to gain new insight into the dynamic neural control of the peripheral circulation. In ten volunteers, we measured HR, ABP, muscle sympathetic nerve activity (MSNA), femoral blood velocity (FBV), and skeletal muscle tissue oxygenation (TO_m) before and during +40 mmHg sinusoidal neck pressure (NP) at 0.1Hz. We performed spectral and cross-spectral analysis of all measurements in the low frequency (LF 0.085-0.115) to quantify the degree of CBR-mediated entrainment and to establish transfer function gain, coherence, and phase relationships. Compared to baseline, sinusoidal NP provoked a significant increase in LF spectral power for R-R interval (RRI), ABP, MSNA, FBV, and TO_m. Transfer function analysis in the LF showed high coherence (>0.5) and a negative phase shift for all measurements; the phase delay was very short for MSNA (-6.1±4.7 degrees) and RRI (-19.4±3.9 degrees), followed by FBV (-30.1±3.6 degrees), ABP (-72.2±6.9 degrees), and finally TO_m (-93.3±11.1 degrees). These data have demonstrated simultaneous entrainment of all CBR efferent measurements, ranging from cardiac chronotropic effects to alterations at the level of the skeletal muscle microcirculation. Furthermore, we provide new insight into CBR signal transduction by defining the temporal relationship between CBR unloading and end-organ responsiveness.

INTRODUCTION

Reflex control of cardiovascular function is by nature designed to maintain adequate arterial blood pressure on a beat-to-beat basis through alterations in heart rate and peripheral vasomotor tone. The carotid baroreceptor component of the arterial baroreflex has been well characterized using techniques that mechanically alter the pressure surrounding the carotid receptors (i.e. variable pressure neck chamber) and observing subsequent changes at the end organ (Parry, 1957; Eckberg *et al.*, 1975; Potts *et al.*, 1993; Ogoh *et al.*, 2002; Fadel *et al.*, 2003). In humans, baroreflex-mediated changes in efferent activity have traditionally been evaluated using measurements of heart rate (HR) (Eckberg, 1977) and arterial blood pressure (ABP) (Mancia *et al.*, 1984), and more recently muscle sympathetic nerve activity (MSNA) (Sanders *et al.*, 1989; Fadel *et al.*, 2001a) and vascular conductance (Ogoh *et al.*, 2002; Keller *et al.*, 2003). While these measurements have elegantly characterized individual end-organ components of the carotid baroreflex (CBR), the direct influence of the CBR on peripheral hemodynamic control has not been established. Moreover, the CBR-mediated changes in HR, ABP, MSNA, and peripheral blood flow have not been assessed collectively to examine the *temporal relationship* between all cardiovascular variables of this complex, closed-loop control system.

Carotid baroreflex testing has traditionally been performed statically, as described by a sigmoid-shaped curve relating R-R interval (RRI), HR, or ABP to carotid sinus transmural pressure following the standard neck pressure (NP) and neck suction (NS) technique (Eckberg, 1980a). However, recent evidence suggests alternate models are

more appropriate to evaluate the true *dynamic* nature of the CBR. First utilized by Bath *et al* (1981), oscillatory changes in neck chamber pressure across several minutes provoke static changes in carotid sinus transmural pressure in a dynamic (i.e. 7.5-sec on, 7.5-sec off) cycle pattern. Oscillatory CBR loading with neck suction has since been applied in studies investigating respiratory sinus arrhythmia (Keyl *et al.*, 2000), the role of the CBR in modulation of cutaneous blood flow (Bernardi *et al.*, 1997), and CBR control of muscle sympathetic outflow (Bath *et al.*, 1981; Furlan *et al.*, 2003). These studies have demonstrated an apparent "entrainment" of RRI, MSNA, and ABP corresponding to the oscillating frequency of the neck chamber pressure, providing direct evidence of dynamic CBR control on these measured variables. However, this dynamic model has not been utilized to assess CBR control of the peripheral circulation. Based on the success of the oscillatory neck suction technique, in the present study we have for the first time introduced sinusoidal NP to create oscillatory sympathoexcitation and assess CBR control of the peripheral circulation.

Repeated oscillations in carotid sinus transmural pressure produced by the neck chamber technique also provide the opportunity for unique modeling of CBR reflex control using spectral analysis techniques. Cross-spectral analysis methods are now widely used to evaluate the transfer function of RRI and ABP variability as an index of both static and dynamic baroreflex gain (Clayton *et al.*, 1995; Sleight *et al.*, 1995). Others have utilized spectral power analysis of RRI, ABP, and MSNA in the frequency domain to estimate autonomic balance in response to pharmacologic (Saul *et al.*, 1990; Nakata *et al.*, 1998) and reflex (Bernardi *et al.*, 1997) changes in carotid sinus pressure. However,

to our knowledge the spectral analysis technique has not been applied to analyze dynamic CBR modulation of hemodynamic control at the level of the skeletal muscle microcirculation using measures of femoral blood velocity (FBV) and skeletal muscle tissue oxygenation (TO_m). Furthermore, cross-spectral analysis of HR, ABP, MSNA, FBV, and TO_m for analysis of the temporal relationship between end-organs has not been considered. Therefore, we sought to determine whether application of sinusoidal NP would provoke concomitant entrainment of HR, ABP, MSNA, FBV, and TO_m in humans, to evaluate the relative degree of CBR control for each effector measurement, and to establish the temporal relationship between these efferent cardiovascular variables.

METHODS

Experimental Protocol

Ten young (18-35 yrs) volunteer subjects free from obvious cardiopulmonary and neuromuscular disease participated in the study. Written informed consent was obtained from all participants, and experiments were approved by the local Institutional Review Board at the University of North Texas Health Science Center and the local ethics committees of Copenhagen and Frederiksberg, Denmark. All subjects were familiarized with the procedures prior to the experimental day, which included evaluation of CBR responsiveness to ensure the variable pressure neck chamber technique could adequately alter carotid sinus transmural pressure (Querry *et al.*, 2001). All studies were performed in a quiet, thermoneutral environment, with subjects in a semi-recumbent position (approximately 30 degrees reclined). Continuous measurements of heart rate (HR),

arterial blood pressure (ABP, finger photoplethysmography), femoral blood velocity (FBV, ultrasound Doppler), and tissue oxygenation (TO_m, near-infrared spectroscopy) were collected. The peroneal nerve was instrumented for continuous recordings of muscle sympathetic nerve activity (MSNA). Following all instrumentation, 5-min data segments were collected before and during 5-sec pulses of neck pressure (NP) at +40 Torr applied in a oscillating sinusoidal manner (0.1 Hz, i.e. 5-secs on, 5-secs off for 5-min) using a traditional neck collar.

TECHNIQUES OF MEASUREMENT

Sinusoidal Neck Pressure Technique. The traditional neck collar technique alters neck chamber pressure using either a pulsatile (R-wave gated) or static stimulus, producing a sigmoid curve relating R-R interval or ABP to carotid sinus pressure (Sleight, 1992). However, recent studies analyzing the dynamic relationship of the CBR suggest that a more sophisticated, non-linear mathematical model may be better suited to describe the intrinsic nature of the system (Zhang *et al.*, 2001). Complex modeling restricts the techniques which can be used to provide CBR loading and unloading, and in the case of spectral analysis a relatively long, steady-state stimulus is required. To meet these criteria, we have introduced a sinusoidal NP technique that may be applied in an oscillating manner over several minutes. This sympathoexcitatory stimulus creates fluctuations in carotid sinus transmural pressure at a pre-determined frequency, and the modifications in efferent CBR activity are then evaluated using end-organ measurements of HR, ABP, MSNA, FBV, and TO_m. We reasoned that the degree of efferent activity

may be quantified by analyzing the variability of the above-mentioned cardiovascular variables in response to sinusoidal NP stimuli.

Ultrasound Doppler. Ultrasound Doppler technology has been used in a number of investigations to accurately determine femoral blood velocity and calculated leg blood flow at rest. (Walloe & Wesche, 1988; Shoemaker *et al.*, 1994; Radegran, 1997b; MacDonald *et al.*, 1998; Saltin *et al.*, 1998; Hoelting *et al.*, 2001). Femoral blood velocity (FBV) was determined at the common femoral artery distal to the inguinal ligament but above the bifurcation into the superficial and profund femoral branch, and was insonated at a fixed perpendicular angle (45 degrees). FBV was measured with a bidirectional Doppler transducer operating at 5 MHz (model MD6, D.E. Hokanson, Inc., Bellevue, WA, USA) and calculated according to the formula $f_a = 64.9V\cos\text{Ø}Hz$, where f_a is the audio frequency, Ø is the angle of insonation, and V is the blood velocity in cm/sec. This procedure of blood velocity measurement has previously been validated and shown to produce accurate absolute values (Radegran, 1997a). Since it has been demonstrated that femoral artery diameter does not change significantly in response to +40 Torr neck pressure (Keller *et al.*, 2003), femoral blood flow was not calculated, and thus power spectral analysis was performed using the raw FBV signal.

Near-Infrared (NIR) Spectroscopy. NIR spectroscopy is based upon the relative ease with which infrared light (700-1000 nm) passes through biological tissue, and on the O_2-dependant absorption changes of hemoglobin and myoglobin. Tissue oxygenation

measurements are limited to the skeletal muscle microcirculation due to the low probability that photons of light will emerge from arteries and veins (Beer's law), and therefore provide a beat-to-beat index of skeletal muscle tissue oxygen delivery relative to its use. For the present study, two fiber optical bundles with an optode separation of 4-cm were placed on the skin over the vastus lateralis muscle, 15-20 cm above the knee along the major axis of the muscle. The probe was secured with adhesive tape and covered with an elastic bandage to shield ambient light and minimize movement artifact. NIR signals at four different wavelengths were sampled concurrently at a rate of 1 Hz, converted to optical densities by using known algorithms, and stored digitally for analysis (NIRO 300, Hamamatsu Photonics, Hamamatsu City, Japan). The NIR HbO_2 signal (expressed in arbitrary units) was used as an index of tissue oxygenation (TO_m) and as an indirect indication of microcirculatory blood flow (Hansen *et al.*, 1996; Sander *et al.*, 2000).

Sympathetic Nerve Recordings. Postganglionic MSNA was recorded with standard microneurographic techniques as described previously (Wallin & Eckberg, 1982). Briefly, a tungsten microelectrode was inserted into the peroneal nerve near the fibular head. The nerve signal was processed by a preamplifier and an amplifier (model 662C-3, Nerve Traffic Analyzer; University of Iowa Bioengineering, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified, and integrated by a resistance-capacitance circuit with a time constant of 0.1 s. MSNA recordings display a pulse-synchronous burst pattern and an increase in burst frequency

with end-expiratory breath holds and Valsalva maneuvers. However, there is no response to arousal or skin stroking. These characteristics were used to discriminate between muscle and skin sympathetic nerve fibers. For baseline comparisons, sympathetic nerve activity was expressed as burst frequency.

Data Analyses.

To evaluate the influence of the CBR on hemodynamic variables, sinusoidal neck pressure (NP) at +40 Torr was produced to evoke sinusoidal oscillations in carotid sinus transmural pressure at a frequency of 0.1 Hz. This dynamic input to the CBR at a constant period is presumably transduced to all efferent variables influenced by the CBR, effectively forcing entrainment of these cardiovascular and hemodynamic variables (figure 1). Such CBR entrainment is quantified at the end organ via the degree of change, i.e. "variability", in the measured signal. End-organ variability associated with CBR entrainment is best analyzed using power spectral analysis, which provides a sensitive measure of variability in the frequency domain, creating a discrete spectral peak at the frequency with which sinusoidal NP was applied. This technique also provides the opportunity for cross-spectral analysis to evaluate the linear and temporal relationship between variables (Saul *et al.*, 1991).

Fast Fourier transformation was performed to calculate spectral power of the R-R interval, ABP, MSNA, FBV, and TO_m time series, as described previously (Cooke *et al.*, 1999). Briefly, beat-to-beat changes in ABP, MSNA, FBV, and TO_m were linearly interpolated and resampled at 5 Hz to convert the unequally spaced beat-to-beat time

series to a uniformly spaced time series for spectral and transfer function analysis. Five minute data sets (1500 samples) were evaluated using a 60-sec window sliding every 10 seconds. These data were detrended, Hanning-filtered, and fast-Fourier transformed to their respective frequency representations. The area under the low frequency (LF, 0.085-0.115 Hz) peak was integrated and averaged for all subjects.

The transfer function (TF) phase, gain, and coherence between neck chamber pressure and ABP, MSNA, FBV, and TO_m was estimated using the cross-spectral method (Saul *et al.*, 1991). The TF analysis reflects the relationship between two measured signals by comparing the amplitude of variability over a specific frequency range. The TF between the two signals was calculated as; $TF = S_{xy}(f) / S_{xx}(f)$, where $S_{xx}(f)$ is the autospectrum of neck chamber pressure and $S_{xy}(f)$ is the cross-spectrum between the autospectrum for neck chamber pressure and the autospectrum for the selected end-organ measurement (Zhang *et al.*, 1998). Transfer function gain values evaluate the relative magnitude between the changes in chamber pressure and the end-organ, while transfer function phase values reflect the relative time relationship between any two measured signals (Saul *et al.*, 1991; Zhang *et al.*, 1998). Using phase delay, the latency from changes in chamber pressure to each signal was calculated according to the equation; *time delay (sec) = phase (deg) / 360 * frequency (Hz).* The assumption of linearity and reliability of the transfer function estimation was confirmed by coherence (COH) testing of LF spectral power, with coherence values >0.50 considered as confirmation of a significant linear relationship between any two measured variables (Saul *et al.*, 1991).

Statistical Analysis.

Because the power spectrum of some measured signals exhibited a skewed distribution, natural logarithm (ln) transformation was performed before statistical testing, as described previously (Bernardi *et al.*, 1997; Cevese *et al.*, 2001). The effect of sinusoidal NP was evaluated after data was natural log transformed, resulting in an estimate of spectral power based on the raw signal variability. For the absolute power spectral values less than 1, ln transformation produced a negative value. Student paired *t-test* (baseline versus sinusoidal NP) analysis was performed to test for a significant main effect of sinusoidal NP on HR, ABP, MSNA, FBF, and TO_m. Coherence (COH) function in the LF (0.085-0.115) was used as a statistical test to confirm significant linearity between measurements for transfer function gain and phase analysis, with coherence values ≥ 0.50 considered significant (as described above). All results are expressed as mean ± standard error and statistical significance was set at $P < 0.05$.

RESULTS

Frequency-domain spectral analysis of the Sinusoidal NP response.

Spectral analysis was performed to identify changes in the variability of each signal in the LF (0.085-0.115 Hz) band during baseline and 0.1 Hz sinusoidal NP conditions. The baseline activity for raw signals and the resulting power spectra for one subject is presented in figure 2a and 2b, respectively. During sinusoidal NP, the sympathoexcitation due to positive neck chamber pressure evoked an increase in efferent activity as measured by changes in RRI, MSNA, FBV, ABP, and TO_m. The visible entrainment of raw signals

and the power spectra for one subject during sinusoidal NP is presented in figure 3a and 3b, respectively. Compared to baseline, sinusoidal NP caused a significant increase in LF spectral power of RRI (5.92±.29 vs. 8.11±0.31 ln ms^2, P<0.001), ABP (-0.15±0.20 vs. 2.11±0.27 ln $mmHg^2$, P<0.001), MSNA (-4.11±1.36 vs. -2.45±1.34 ln AU^2, P=0.001), FBV (-10.08±0.44 vs. -7.69±0.73 ln AU^2, P<0.001), and TO_m (-6.38±0.55 vs. -4.73±0.56 ln mV^2, p<0.001), see figure 4.

Cross-spectral transfer function analysis.

Cross-spectral analysis was performed to identify the relationship between variables and address the temporal aspects of the efferent responses. Based on the modeled sequence of signal transduction (see figure 1), we performed transfer function analysis using neck chamber pressure (CP) as it is the only open-loop "input" to the carotid baroreceptors, and thus all transfer function analysis was arranged accordingly; CP-RRI, CP-ABP, CP-MSNA, CP-FBV, and CP-TO_m. During baseline conditions, LF (0.085-0.115 Hz) coherence values were well below 0.5 for all cross-spectral comparisons, suggesting that no significant linear relationship exists in the LF range. In contrast, during sinusoidal NP, LF COH was >0.50 for all signals, allowing evaluation of transfer function gain and phase (table 1). All phase measurements lagged behind CP changes, i.e. all were negative phase shifts (figure 5). The phase delay for RRI was established after subtraction of one half-period (i.e. subtraction of 180 degrees) to ensure that the RRIs preceding CP changes were not used in the calculation, as described previously (Keyl *et al.*, 2000; Keyl *et al.*, 2001). The phase delay was very short for MSNA (-6.1±4.7 degrees or -291±138 ms),

and RRI (-19.4±3.9 degrees or -537±109 ms), followed by FBV (-30.1±3.6 degrees or -965±70 ms) and then ABP (-72.2±6.9 degrees or 1909±192 ms), and TO_m (-93.3±11.1 degrees or -2742±376 ms).

DISCUSSION

Using sinusoidal NP as a dynamic sympathoexcitatory stimulus, the present study has demonstrated simultaneous entrainment of all CBR efferent measurements, ranging from cardiac chronotropic effects to alterations at the level of the skeletal muscle microcirculation. Furthermore, we have established the influence of the CBR on peripheral hemodynamic control through beat-to-beat modulation of skeletal muscle perfusion and tissue oxygenation. Finally, using cross-spectral analysis we have established the sequential relationship between CBR unloading and end-organ responses, providing new information regarding temporal aspects of CBR signal transduction to the peripheral circulation during dynamic sympathoexcitation.

Simultaneous entrainment of cardiovascular variables.

The present study has for the first time identified the simultaneous control of HR, ABP, MSNA, FBV, and TO_m following CBR-mediated sympathoexcitation. These findings extend previous studies aimed at evaluation of individual components of the efferent CBR response (Parry, 1957; Eckberg *et al.*, 1975; Potts *et al.*, 1993; Ogoh *et al.*, 2002; Fadel *et al.*, 2003). Early studies in humans (Bevegard & Shepherd, 1966) identified direct CBR control of RRI and ABP, while more recent studies have begun to consider

CBR control of the peripheral circulation (Keller *et al.*, 2003). MSNA has been measured using the variable pressure neck chamber to provide an index of CBR-mediated alterations in sympathetic outflow to the skeletal muscle vasculature (Sundlof & Wallin, 1978; Wallin & Eckberg, 1982; Rea & Eckberg, 1987; Fadel *et al.*, 2001a). Recently, beat-to-beat ultrasound Doppler measurements have been made in humans to demonstrate changes in femoral blood flow (FBF) and femoral vascular conductance (FVC) following neck pressure (NP) or neck suction (NS), providing direct evidence for CBR control of the peripheral circulation (Keller *et al.*, 2003). However, FVC calculations are based on blood flow measurements taken in large, conduit vessels and do not necessarily reflect blood flow in the skeletal muscle microcirculation, where blood flow is heterogeneous. In the present study, inclusion of tissue oxygenation (TO_m) offers unique insight into CBR control by providing an index of oxygen delivery to the microcirculation, allowing an estimate of microcirculatory blood flow (Hansen *et al.*, 2000b). The observed entrainment of all measurements during sinusoidal NP demonstrate that the CBR exerts concomitant, wide-ranging control at all levels of the cardiovascular system, extending from cardiac chronotropic effects to the alterations at the level of the skeletal muscle microcirculation.

Sinusoidal NP technique and evaluation of dynamic CBR control.

Previous studies investigating CBR function have applied NS and NP as a single 5-sec pulse and assessed the response to this static change in carotid sinus pressure (Eckberg, 1977; Potts *et al.*, 1993; Fadel *et al.*, 2001a; Ogoh *et al.*, 2003a). While providing

pertinent information regarding the cardiovascular response to CBR loading and unloading, this technique does not evaluate the dynamic characteristics of CBR control. Zhang *et al* (2001) have developed a more dynamic model of CBR control that applies dynamic system analysis to evaluate the relationship between HR and ABP changes following acute hypotension. In a recent study, Parati *et al* (2001) proposed the evaluation of spontaneous changes in RRI and ABP as a more appropriate technique to evaluate dynamic baroreflex control, analyzed using time-domain and spectral analysis approaches.

Based on the concepts put forth in these studies, in the present study we have introduced the oscillatory neck pressure technique, which utilizes the classic carotid baroreceptor unloading in a dynamic 0.1Hz pattern across several minutes. Oscillatory neck suction (carotid baroreceptor loading) has been applied in previous studies investigating respiratory sinus arrhythmia (Keyl *et al.*, 2000), the role of the CBR in modulation of cutaneous blood flow (Bernardi *et al.*, 1997) and CBR control of muscle sympathetic outflow (Bath *et al.*, 1981; Furlan *et al.*, 2003). However, considering that increases of sympathetic activity during NP may be greater than reductions of sympathetic activity during NS (Rea & Eckberg, 1987; Keller *et al.*, 2003), we believe sinusoidal NP is a more appropriate technique to effectively measure changes in the peripheral circulation. This robust sympathoexcitatory signal increases the likelihood of a detectable measurement at the microcirculation, where natural dampening may occur due to the inherent physical properties of the vasculature (i.e. windkessel effect). Furthermore, the application of an oscillatory CBR stimulus for five minutes provides the

advantage of an average estimation of the CBR response and allows evaluation of the non-linear characteristics of the system.

Spectral analysis and hemodynamic control.

Frequency-domain spectral analysis provided a unique means for quantifying the degree of CBR control over each measured variable, with the supposition that more tightly regulated measurements create a larger spectral power. A progressive reduction in the LF power spectra is evident as the end-organ measurements are viewed together (see figure 4), suggesting that the influence of the CBR is dampened in the peripheral microcirculation. This observation may be attributed to the physical properties of the vasculature, since the TO_m measurement is an estimate of relative changes in microcirculatory perfusion, downstream from arterial resistance vessels. Nonetheless, a discreet spectral peak is evident for the TO_m signal, which in conjunction with the FBV measurement estimates CBR control on both "sides" of the resistance vessels. Together with MSNA measurements, this novel approach provides insight into the signal transduction of efferent CBR outflow to the skeletal muscle microcirculation.

Temporal aspects of CBR signal transduction.

In conjunction with the spectral power for each measurement, cross-spectral comparison of each measurement against the CP "input" has provided insight into the sequence of events as the sympathoexcitatory stimuli is transduced through the efferent limb of the CBR to the various end-organs (see figure 1). Although transfer function analysis may

theoretically be performed between any two measurements, we reasoned that analysis with CP against the remaining measurements would prevent the confounding effect of negative feedback influence from the previous pulse of NP during the 5-min sinusoidal NP period. For all measurements, LF coherence values were well above the *a priori* significance criteria of 0.5, indicating a stable relationship between input and output and providing further quantitative evidence of CBR-mediated entrainment of skeletal muscle hemodynamic measurements.

Transfer function gain (TFG) provided a quantitative index of the ratio between changes in neck chamber pressure and end-organ measurements in the LF (0.085-0.115). While the TFG between RRI and ABP has been reported previously as an index of baroreflex gain (Saul *et al.*, 1991), TFG has not been reported for measurements of the peripheral circulation. Similar to the LF power spectra, LF TFG values indicate a diminished but discernable relationship between CBR unloading and measurements of MSNA, FBV, and TO_m (see table 1), suggesting a substantial degree of CBR influence at the level of the peripheral microcirculation.

The present study has also delineated the time delay following sinusoidal NP as the efferent nerve activity is measured first by changes in RRI and MSNA, followed by alterations in vasomotion which lead to changes in ABP, FBV and finally TO_m. Previous attempts to characterize this time delay using direct sinus nerve stimulation (Borst & Karemaker, 1983), mathematical modeling (deBoer *et al.*, 1987) and more recently oscillatory neck suction (Keyl *et al.*, 2001) have reported a baroreflex latency of 500-600ms, and our findings for calculated RRI phase latency agree with these values.

However, few studies have attempted to identify temporal aspects of the peripheral blood flow response, though an allusion to the "sluggishness" of the peripheral vascular beds appears in the discussion of early studies utilizing the oscillatory NS technique (Bath *et al.*, 1981). Our findings agree with this hypothesis, showing a progressively increasing phase delay as CBR-mediated efferent activity moves from the carotid sinus to the peripheral vasculature (see figure 5). These data identify a sequential delay in signal transduction as sympathetic activity is recorded first at the peroneal nerve and heart, transduced to the vasculature, and after approximately 700 ms measured via changes in ABP and FBV. A final delay of almost 2 seconds was observed between changes in FBV and TO_m, which may be associated with the transit time for oxygen from the microcirculatory vessels to the muscle tissue. To our knowledge, this is the first report of the time delay associated with signal transduction from the carotid baroreceptor to the skeletal muscle microcirculation in humans. The stability of this phase shift (verified by high coherence values) across an extended (5-min) period of measurement confirms phase and TFG calculations, and further emphasizes the role of the CBR as a key component in the dynamic control of the peripheral circulation.

Potential limitations.

One of the potential limitations of the present study regards the use of near-infrared spectroscopy as an index of microcirculatory blood flow. NIRS does not directly measure tissue blood flow, but instead provides a qualitative index of tissue oxygenation. Recent studies indicate that the contribution of myoglobin (Seiyama *et al.*, 1988; Mancini *et al.*,

1994) and skin blood flow (Hampson & Piantadosi, 1988; Mancini *et al.*, 1994) to the NIRS oxygenation signal is minimal, and that NIRS measurements are highly reproducible (Bhambhani *et al.*, 2001). Others have demonstrated that under steady-state conditions, NIR spectroscopy measurements may be used to detect decreases in oxygen supply, such as the decrease that occurs during sympathetically induced vasoconstriction (Mancini *et al.*, 1994; Hansen *et al.*, 1996; Hansen *et al.*, 2000a). In addition, other studies have demonstrated a close correlation between blood flow values measured by plethysmography, the Fick method, and NIRS (Edwards *et al.*, 1993; Van Beekvelt *et al.*, 2001). Recent validation studies report a high correlation between NIRS and conventional ultrasound Doppler measurements in rat hind limb (Fadel *et al.*, 2002) and human forearm (Keller, 2003). Together, these studies support the use of TO_m as an indirect index of microcirculatory blood flow in the present study.

CONCLUSION

The present study has demonstrated for the first time the dynamic CBR-mediated entrainment of HR, ABP, MSNA, FBV, and TO_m in humans. This observed entrainment of all measurements during sinusoidal NP demonstrated that the CBR exerts concomitant, wide-ranging control at all levels of the cardiovascular system. Furthermore, by using simultaneous measurements of ultrasound Doppler and near-infrared spectroscopy, we were able to identify the CBR as a key component in the dynamic control of the peripheral circulation. Finally, through cross-spectral analysis we have established the temporal relationship between CBR unloading and end-organ responsiveness, extending

from cardiac chronotropic effects to alterations at the level of the skeletal muscle microcirculation, providing new insights into CBR signal transduction.

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TABLE 1 - Frequency domain results from cross-spectral analysis.

		BASELINE			SINUSOIDAL NP		
		LF TFG	LF COH	LF PHASE	LF TFG	LF COH	LF PHASE
CP-RRI	(mmHg/ms)	---	<0.5	---	3.111±0.621	0.78±0.05	-19±3.9
CP-ABP	(mmHg/mmHg)	---	<0.5	---	0.135±0.033	0.77±0.04	-69±6.9
CP-MSNA	(mmHg/mV)	---	<0.5	---	0.043±0.015	0.61±0.07	-11±4.9
CP-FBV	(mmHg/mV)	---	<0.5	---	0.002±0.000	0.86±0.02	-35±2.5
CP-TOm	(mmHg/mV)	---	<0.5	---	0.006±0.002	0.75±0.05	-99±13.5

Results from cross-spectral analysis between chamber pressure (CP) and the end-organ measurements of R-R interval (RRI), arterial blood pressure (ABP), muscle sympathetic nerve activity (MSNA), femoral blood velocity (FBV), and tissue oxygenation (TOm). Transfer function gain (TFG), coherence (COH), and phase were calculated in the low frequency (LF, 0.085-0.115) during baseline and sinusoidal NP conditions. All LF coherence values fell well below 0.5 in the baseline condition, indicating no significant linear relationship between measurements. Values are mean ± standard error.

FIGURE LEGEND

FIGURE 1: Control system model for carotid baroreflex (CBR) influence on cardiac and hemodynamic responses. Sinusoidal neck pressure (NP) provides an external input to the carotid baroreceptors, which sense an error signal and modify efferent output in an attempt to return the system to the homeostatic set point. Note the closed-loop relationship, with arterial blood pressure providing negative feedback. *Abbreviations:* CBR=carotid baroreflex, RRI=R-R interval, ABP=arterial blood pressure, MSNA=muscle sympathetic nerve activity, FBV=femoral blood velocity, TO_m=tissue oxygenation.

FIGURE 2: Individual tracing of beat-to-beat measurements during baseline (panel A) and the power spectra derived from baseline measurements (panel B). No spectral peak is evident in the LF (0.085-0.115) band.

FIGURE 3: Individual tracing of beat-to-beat measurements during 0.1 Hz sinusoidal neck pressure (panel A) and the power spectra derived from the 5-min Sinusoidal NP period (panel B). The 0.1 Hz oscillations neck chamber pressure created a clear entrainment of all measurements, which produced a discreet spectral peak in the LF.

FIGURE 4: LF spectral power of all measurements during baseline (black bars) and during sinusoidal NP (grey bars) after natural log (ln) data transformation. Sinusoidal NP produced a significant increase in LF power of all signals, confirming carotid baroreflex

control of all hemodynamic measurements. (*) Significantly different than baseline, $P<0.01$.

FIGURE 5: Phase analysis revealed a clear latency from chamber pressure (CBR input) to changes in RRI, MSNA, FBV, ABP, and TO_m. This phase delay (left axis) reflects the time lag (right axis) that exists as the efferent sympathoexcitation is transduced from the carotid baroreceptor to the skeletal muscle microcirculation.

FIGURE 1

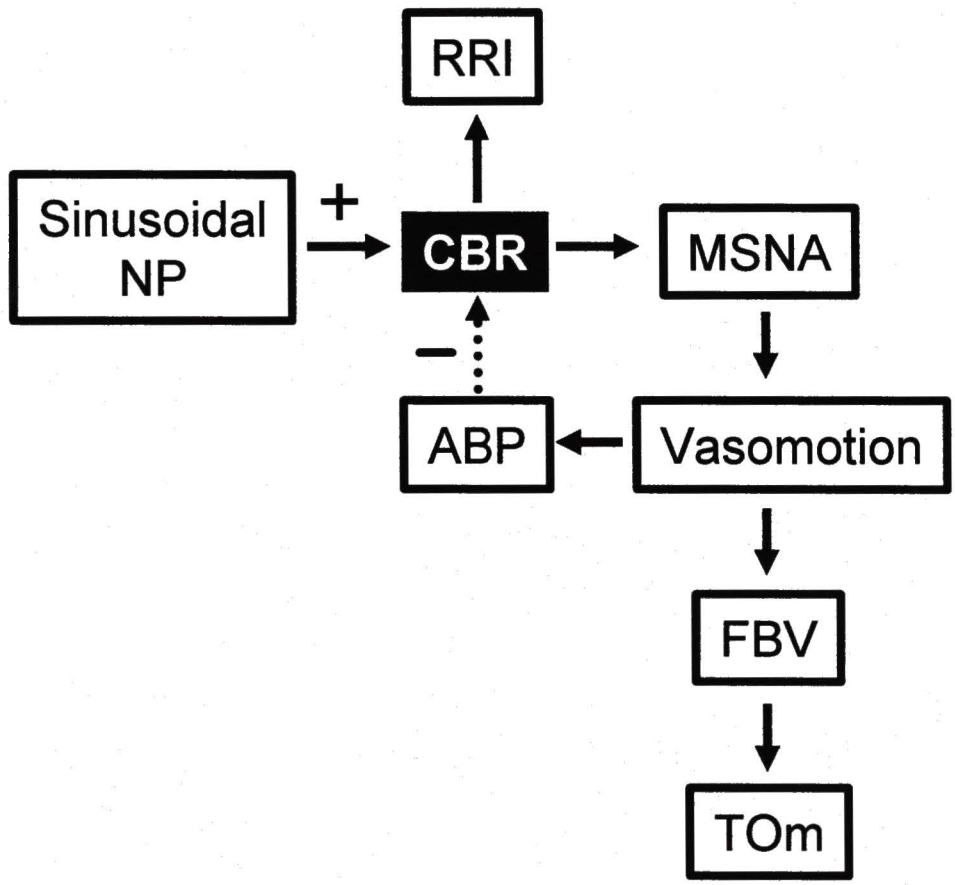


FIGURE 2a

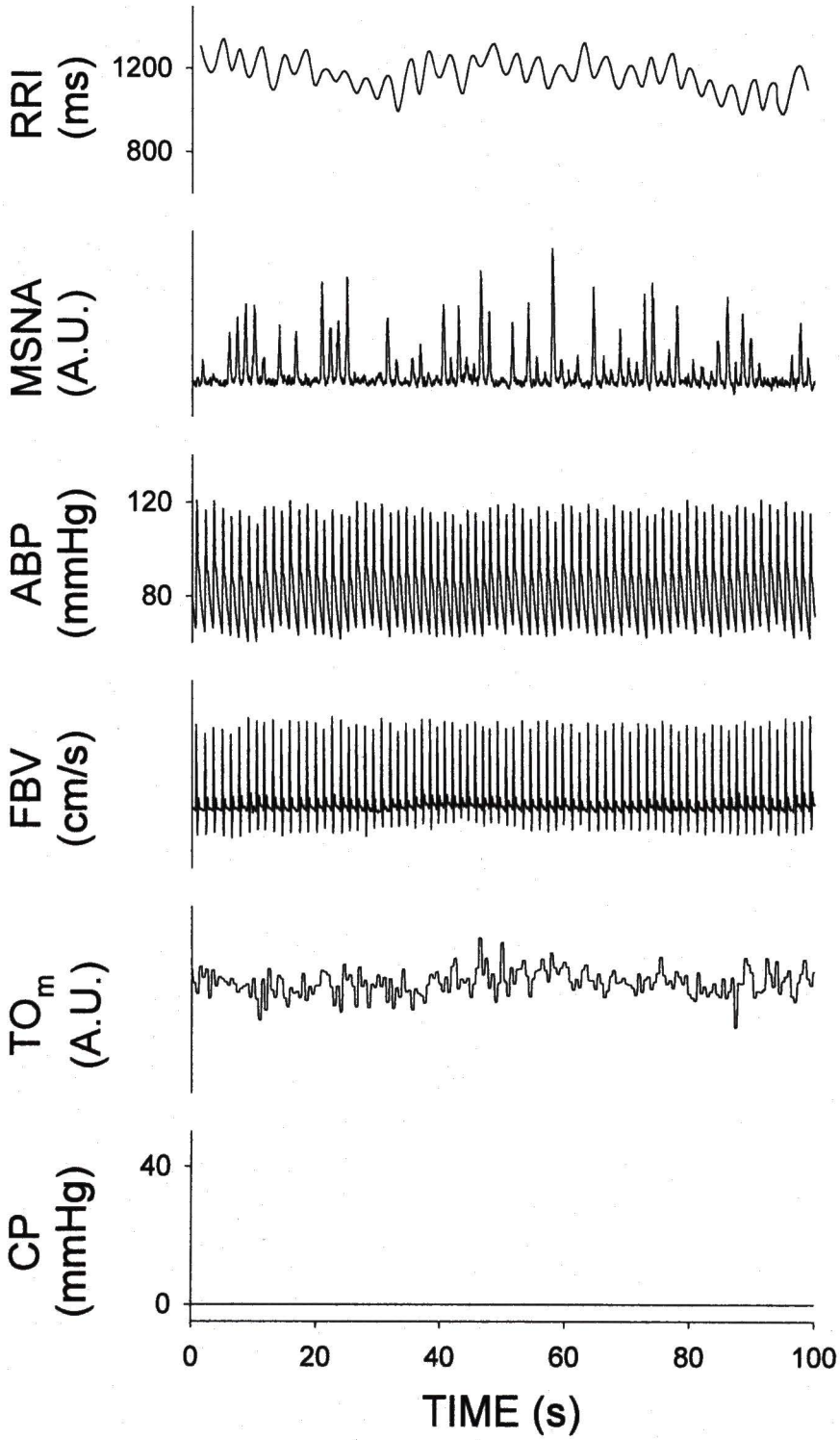

RRI (ms)
1200
800
MSNA (A.U.)
ABP (mmHg)
120
80
FBV (cm/s)
TOm (A.U.)
CP (mmHg)
40
0
0
20
40
60
80
100
TIME (s)

FIGURE 2b

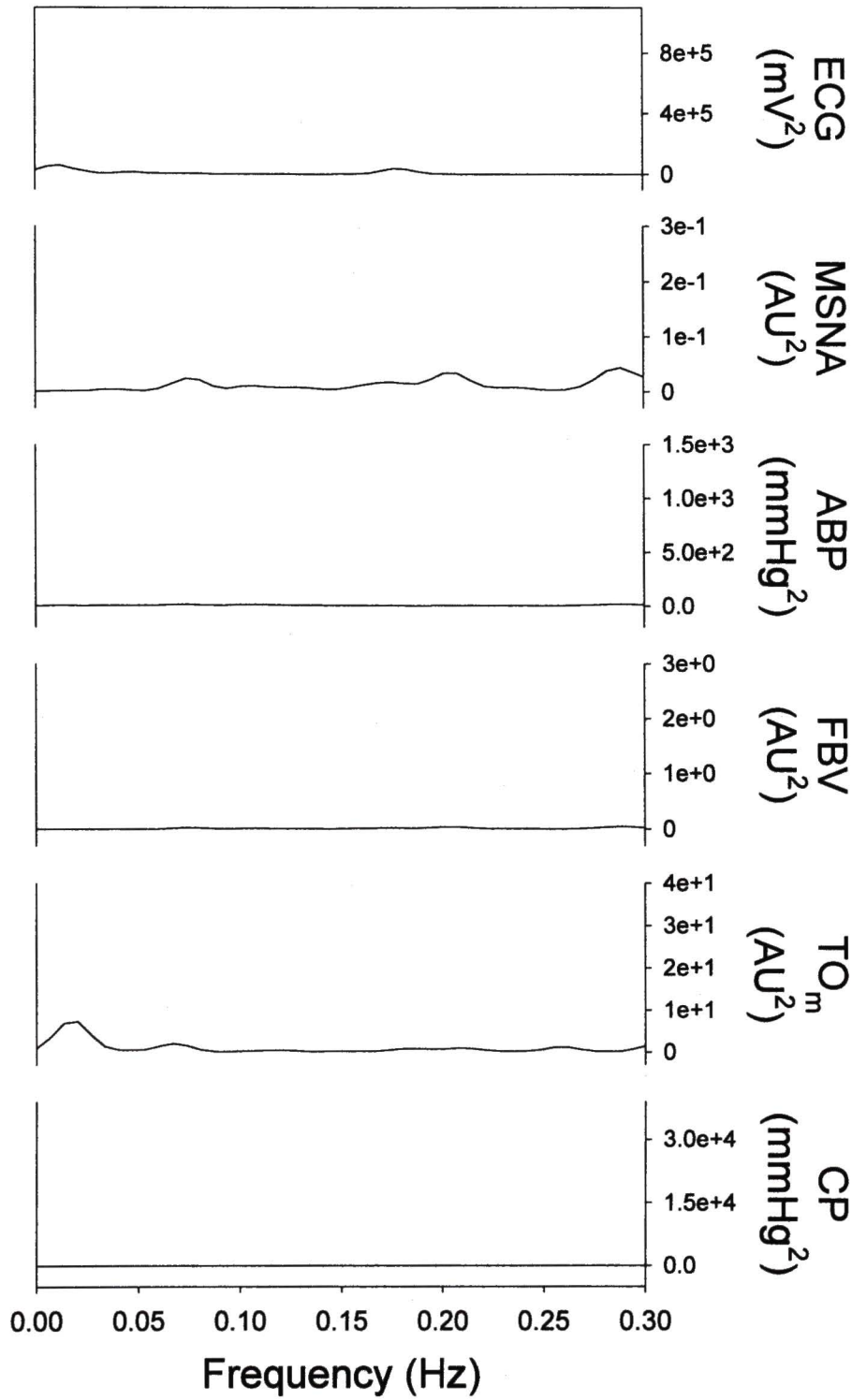

ECG
(mV²)
8e+5
4e+5
0
MSNA
(AU²)
3e-1
2e-1
1e-1
0
ABP
(mmHg²)
1.5e+3
1.0e+3
5.0e+2
0.0
FBV
(AU²)
3e+0
2e+0
1e+0
0
TOm
(AU²)
4e+1
3e+1
2e+1
1e+1
0
CP
(mmHg²)
3.0e+4
1.5e+4
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Frequency (Hz)

FIGURE 3a

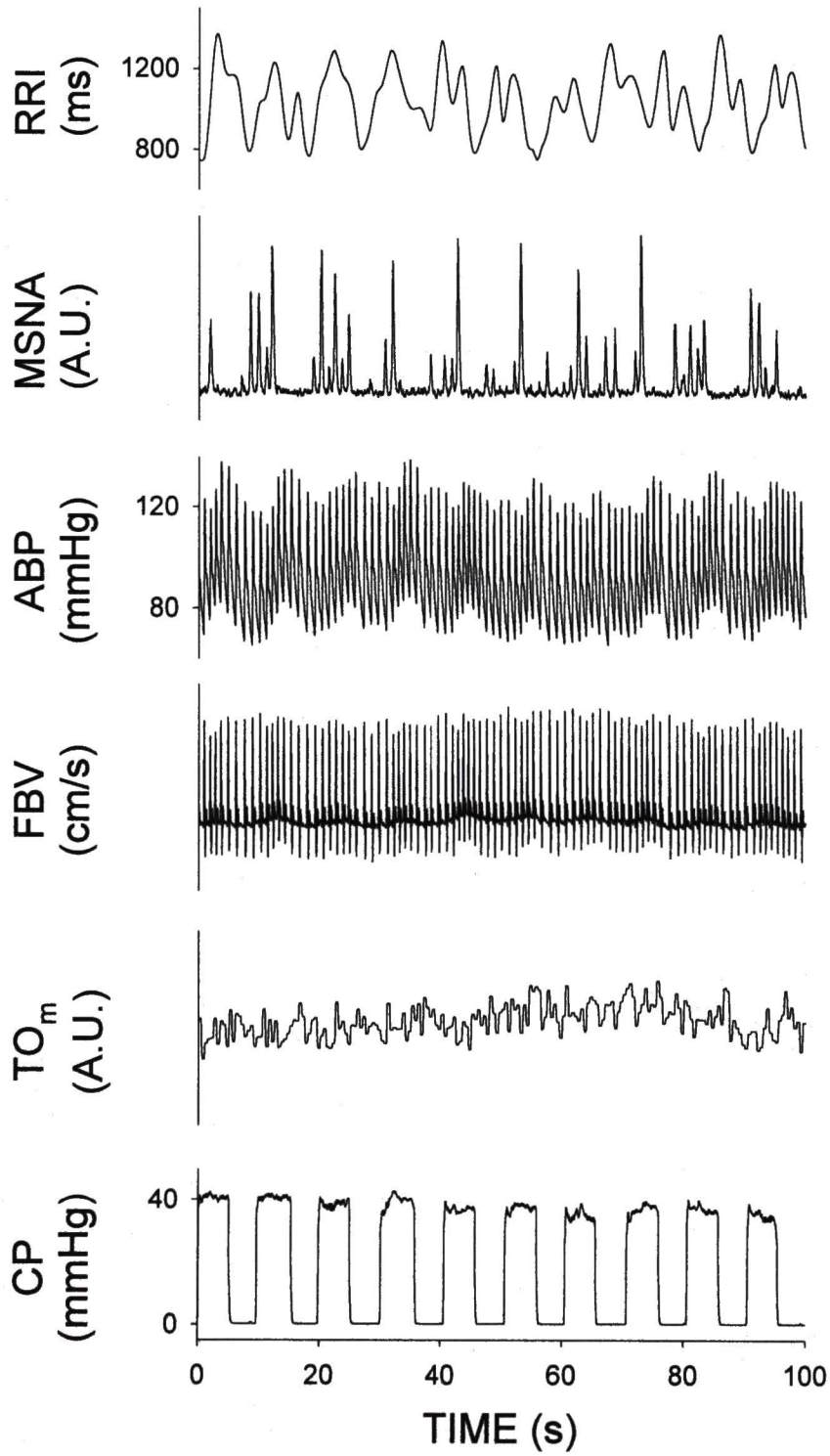

RRI (ms)
1200
800
MSNA (A.U.)
ABP (mmHg)
120
80
FBV (cm/s)
TOm (A.U.)
CP (mmHg)
40
0
0
20
40
60
80
100
TIME (s)

FIGURE 3b

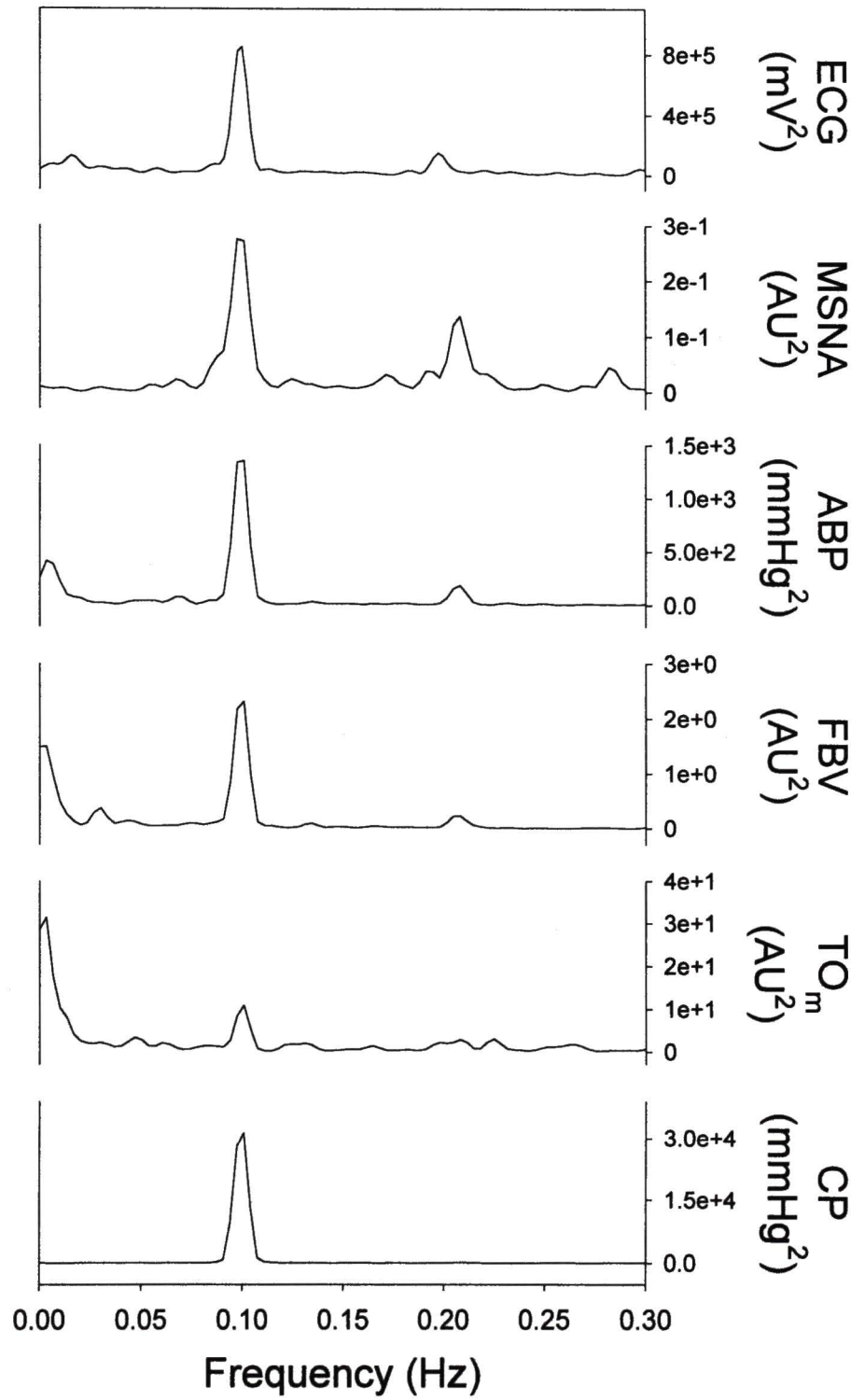

ECG (mV²)
8e+5
4e+5
0
MSNA (AU²)
3e-1
2e-1
1e-1
0
ABP (mmHg²)
1.5e+3
1.0e+3
5.0e+2
0.0
FBV (AU²)
3e+0
2e+0
1e+0
0
TOm (AU²)
4e+1
3e+1
2e+1
1e+1
0
CP (mmHg²)
3.0e+4
1.5e+4
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Frequency (Hz)

FIGURE 4

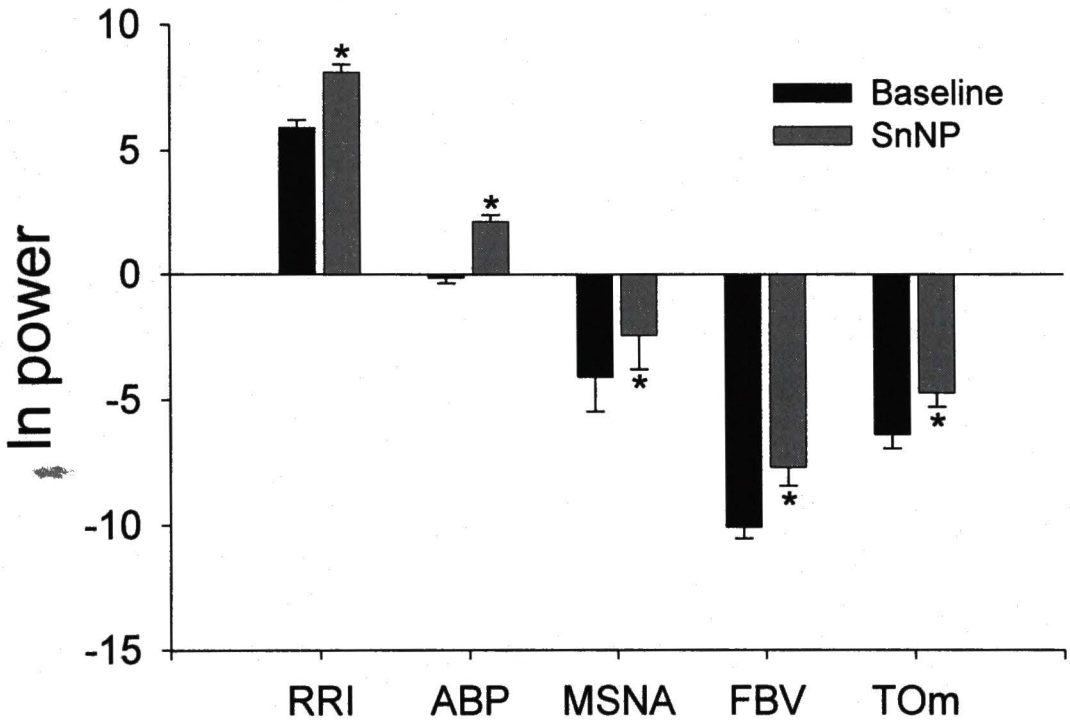
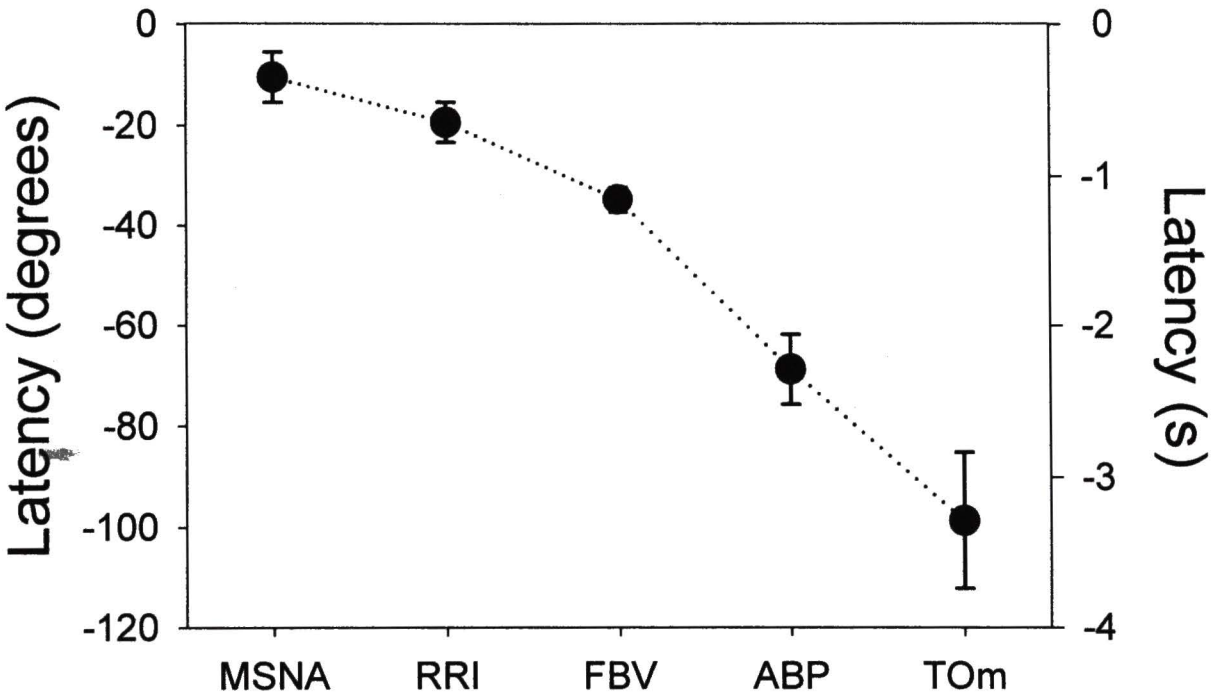


FIGURE 5


0
-20
-40
-60
-80
-100
-120
Latency (degrees)
0
-1
-2
-3
-4
Latency (s)
MSNA
RRI
FBV
ABP
TOm

CHAPTER IV

Reflex versus Local Control of the Peripheral Circulation During Dynamic Exercise in Humans.

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Running head: Carotid baroreflex control during exercise

Key Words: Near-infrared spectroscopy; Knee-extensor exercise; Carotid baroreflex

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SUMMARY

We sought to determine the relationship between carotid baroreflex (CBR)-mediated control and local control of the skeletal muscle vasculature during dynamic exercise. In twelve subjects (18-35 yrs), oscillatory NP (+40 Torr) was applied at 0.1 Hz (i.e. 5-sec on, 5-sec off) for five minutes to determine the degree of CBR control over heart rate (HR), arterial blood pressure (ABP), muscle sympathetic nerve activity (MSNA), femoral blood velocity (FBV), and skeletal muscle tissue oxygenation (TO_m) at rest and during 7W dynamic knee-extension exercise. TO_m measurements on both the exercising (EL) and non-exercising leg (NEL) were evaluated. Fast Fourier transformation was performed on 5-min segments to calculate spectral power of the R-R interval, ABP, MSNA, FBV, and TO_m time series. The low-frequency (LF, 0.085-0.115) power spectra were compared to evaluate the degree of CBR control of end-organ measurements. At rest, sinusoidal NP significantly increased LF spectral power of RRI, ABP, MSNA (n=6), and FBV. During exercise, the change in spectral power from baseline to sinusoidal NP was similar to rest for RRI and MSNA, while CBR-mediated changes in ABP and FBV were attenuated. Changes in spectral power of TO_m during sinusoidal NP were similar between the EL and NEL at rest. However, during exercise the changes in TO_m power were significantly less in the EL, while changes in the NEL were similar to rest. We conclude that exercise attenuates CBR control of peripheral hemodynamics, suggesting a shift towards more predominant local control over the exercising muscle vasculature, but without complete abolition of systemic ABP control.

INTRODUCTION

During exercise, the vasculature of active skeletal muscle is influenced by the opposing effects of neural sympathetic input and locally-produced vasodilatory metabolites. In 1962, Remensnyder *et al.* coined the term "functional sympatholysis" to describe the observation that sympathetic vasoconstriction in active skeletal muscles may be overridden by local control factors (Remensnyder, 1962). Many studies in animals (Thomas *et al.*, 1998; Buckwalter *et al.*, 2001; Dinenno & Joyner, 2003) and humans (Hansen *et al.*, 1999; Dinenno & Joyner, 2003; Rosenmeier *et al.*, 2003) have considered the influence of metabolic factors on sympathetic control of the skeletal muscle vasculature during exercise. However, the interaction of this sympatholytic event with *reflex* arterial blood pressure (ABP) control mechanisms remains unclear.

The importance of local metabolic events on ABP regulation is underscored by the fact that as blood flow in the exercising muscle increases, changes in vascular conductance in the skeletal muscle vascular bed produce more pronounced systemic effects (Buckwalter & Clifford, 2001; Collins *et al.*, 2001). Thus, effective reflex control of the exercising muscle vasculature is crucial to ensure adequate tissue perfusion is achieved without sacrificing systemic arterial pressure. It is well established that carotid baroreflex (CBR) control of R-R interval (RRI) (Eckberg, 1977) and ABP (Bevegard & Shepherd, 1966; Potts *et al.*, 1993) is preserved from rest to exercise in humans. More recently, continued CBR control of MSNA (Fadel *et al.*, 2001a) and leg vascular conductance (Keller *et al.*, 2003) during exercise have also been demonstrated. Using the variable pressure neck chamber, these studies have established the cardiac and

hemodynamic responsiveness to a single, static pulse of neck pressure (NP, CBR unloading) and neck suction (NS, CBR loading). However, the extent of CBR control at the level of the skeletal muscle microcirculation in the face of exercise-induced local metabolic influences has not been investigated.

It is well known that the CBR is by nature a dynamic system with intrinsic nonlinearity (Eckberg, 1980b; Zhang *et al.*, 2001). As such, recent evidence suggests alternate models may be more appropriate to evaluate the *dynamic* nature of the CBR, using more prolonged, dynamic stimuli such as oscillatory NS (Bernardi *et al.*, 1997; Keyl *et al.*, 2000; Zhang *et al.*, 2001). To our knowledge, CBR-mediated control of cardiovascular function during oscillatory CBR unloading (NP) has not been demonstrated during dynamic exercise. Evaluation of the dynamic properties of CBR control with oscillatory NP may be achieved using nonlinear frequency domain analysis. With this technique, unique modeling of reflex control is possible by providing an oscillating input to the CBR (i.e. sinusoidal NP) and measuring changes at the end-organ using spectral analysis of signal variability. In addition, cross-spectral methods allow evaluation of the linear relationship between measurements using the transfer function technique, now widely used as an estimate of baroreflex gain (Clayton *et al.*, 1995; Sleight *et al.*, 1995) and autonomic balance (Saul *et al.*, 1990; Bernardi *et al.*, 1997; Nakata *et al.*, 1998). However, the spectral analysis technique has not been applied to analyze dynamic CBR modulation of hemodynamic control at the level of the skeletal muscle microcirculation using measures of femoral blood velocity (FBV) and skeletal muscle tissue oxygenation (TO_m). These end-organ measurements are of particular

interest, since exercise-induced metabolites are known to profoundly influence vascular control during exercise.

In the present study we sought to determine the relationship between CBR-mediated reflex control and local metabolic control of the skeletal muscle vasculature during dynamic exercise. Oscillatory NP was applied to determine the degree of CBR control over hemodynamic function at rest and during moderate-intensity knee extension exercise. We hypothesized that dynamic CBR-mediated sympathoexcitation would produce similar changes in RRI, ABP, and MSNA at rest and during exercise, but that metabolites from the exercising muscle would attenuate CBR control of peripheral hemodynamic measurements of FBV and TO_m.

METHODS

Experimental Protocol

Twelve young (18-35 yrs) volunteer subjects free from obvious cardiopulmonary and neuromuscular disease participated in the study. Written informed consent was obtained from all participants, and experiments were approved by the local Institutional Review Board at the University of North Texas Health Science Center. All subjects were familiarized with the procedures prior to the experimental day, which included evaluation of CBR responsiveness to ensure the variable pressure neck chamber technique could adequately alter carotid sinus transmural pressure (Querry *et al.*, 2001). All studies were performed in a quiet, thermoneutral environment, with subjects in a semi-recumbent position (approximately 30 degrees reclined). Continuous measurements of heart rate

(HR), arterial blood pressure (ABP, finger photoplethysmography), femoral blood velocity (FBV, ultrasound Doppler), and tissue oxygenation (TO_m, near-infrared spectroscopy) were collected. The peroneal nerve of the non-exercising leg was instrumented for continuous recordings of muscle sympathetic nerve activity (MSNA, n=6). Following all instrumentation, 5-min data segments were collected before and during 5-sec pulses of neck pressure (NP) at +40 Torr applied in a oscillating sinusoidal manner (0.1 Hz, i.e. 5-secs on, 5-secs off for 5-min) using a traditional neck collar. After resting measurements, unilateral knee-extension exercise was performed at 30 kicks per minute (7W) on a modified cycle ergometer, as described previously (Andersen *et al.*, 1985). After five minutes of exercise to achieve steady-state conditions, sinusoidal NP was again applied for 5-min, followed by 5-min of steady-state exercise measurements.

TECHNIQUES OF MEASUREMENT

Sinusoidal Neck Pressure Technique. The traditional neck collar technique alters neck chamber pressure using either a pulsatile (R-wave gated) or static stimulus, producing a sigmoid curve relating R-R interval or ABP to carotid sinus pressure (Sleight, 1992). However, recent studies analyzing the dynamic relationship of the CBR suggest that a more sophisticated, nonlinear mathematical model may be better suited to describe the intrinsic nature of the system (Zhang *et al.*, 2001). Complex modeling restricts the techniques which can be used to provide CBR loading and unloading, and in the case of spectral analysis a relatively long, steady-state stimulus is required. To meet these criteria, we have utilized a sinusoidal NP technique that may be applied in an

oscillating manner over several minutes. This sympathoexcitatory stimulus creates fluctuations in carotid sinus transmural pressure at a pre-determined frequency, and the modifications in efferent CBR activity are then evaluated using end-organ measurements of HR, ABP, MSNA, FBV, and TO_m. We reasoned that the degree of efferent activity may be quantified by analyzing the variability of the above-mentioned cardiovascular variables in response to sinusoidal NP stimuli.

Ultrasound Doppler. Ultrasound Doppler technology has been used in a number of investigations to accurately determine femoral blood velocity and calculated leg blood flow at rest. (Walloe & Wesche, 1988; Shoemaker *et al.*, 1994; Radegran, 1997b; MacDonald *et al.*, 1998; Saltin *et al.*, 1998; Hoelting *et al.*, 2001). Femoral blood velocity (FBV) was determined at the common femoral artery distal to the inguinal ligament but above the bifurcation into the superficial and profund femoral branch, and was insonated at a fixed perpendicular angle (45 degrees). FBV was measured with a bidirectional Doppler transducer operating at 5 MHz (model MD6, D.E. Hokanson, Inc., Bellevue, WA, USA) and calculated according to the formula $f_a = 64.9V\cos\text{Ø}Hz$, where f_a is the audio frequency, Ø is the angle of insonation, and V is the blood velocity in cm/sec. This procedure of blood velocity measurement has previously been validated and shown to produce accurate absolute values (Radegran, 1997a). It has been demonstrated that femoral artery diameter does not change significantly in response to +40 Torr neck pressure (Keller *et al.*, 2003) or during 7W knee-extensor exercise (Wray *et al.* 2003, in

press), and in the present study we confirm these findings. Thus, femoral blood flow was not calculated, and power spectral analysis was performed using the raw FBV signal.

Near-Infrared (NIR) Spectroscopy. NIR spectroscopy is based upon the relative ease with which infrared light (700-1000 nm) passes through biological tissue, and on the O_2-dependant absorption changes of hemoglobin and myoglobin. Tissue oxygenation measurements are limited to the skeletal muscle microcirculation due to the low probability that photons of light will emerge from arteries and veins (Beer's law), and therefore provide a beat-to-beat index of skeletal muscle tissue oxygen delivery relative to its use. For the present study, two fiber optical bundles with an optode separation of 4-cm were placed on the skin over the vastus lateralis muscles of both legs, 15-20 cm above the knee along the major axis of the muscle. The probe was secured with adhesive tape and covered with an elastic bandage to shield ambient light and minimize movement artifact. NIR signals at four different wavelengths were sampled concurrently at a rate of 1 Hz, converted to optical densities by using known algorithms, and stored digitally for analysis (NIRO 300, Hamamatsu Photonics, Hamamatsu City, Japan). The NIR HbO_2 signal (expressed in arbitrary units) was used as an index of tissue oxygenation (TO_m) and as an indirect indication of microcirculatory blood flow (Hansen *et al.*, 1996; Sander *et al.*, 2000).

Sympathetic Nerve Recordings. Postganglionic MSNA was recorded with standard microneurographic techniques as described previously (Wallin & Eckberg, 1982).

Briefly, a tungsten microelectrode was inserted into the peroneal nerve near the fibular head. The nerve signal was processed by a preamplifier and an amplifier (model 662C-3, Nerve Traffic Analyzer; University of Iowa Bioengineering, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified, and integrated by a resistance-capacitance circuit with a time constant of 0.1 s. MSNA recordings display a pulse-synchronous burst pattern and an increase in burst frequency with end-expiratory breath holds and Valsalva maneuvers. However, there is no response to arousal or skin stroking. These characteristics were used to discriminate between muscle and skin sympathetic nerve fibers. For baseline comparisons, sympathetic nerve activity was expressed as burst frequency.

Data Analyses.

To evaluate the influence of the CBR on hemodynamic variables, sinusoidal neck pressure (NP) at +40 Torr was produced to evoke sinusoidal oscillations in carotid sinus transmural pressure at a frequency of 0.1 Hz. This dynamic input to the CBR at a constant period is presumably transduced to all efferent variables influenced by the CBR, effectively forcing entrainment of these cardiovascular and hemodynamic variables. Such CBR entrainment is quantified at the end organ via the degree of change, i.e. "variability", in the measured signal. End-organ variability associated with CBR entrainment is best analyzed using power spectral analysis, which provides a sensitive measure of variability in the frequency domain, creating a discrete spectral peak at the frequency with which sinusoidal NP was applied. This technique also provides the

opportunity for cross-spectral analysis to evaluate the linear and temporal relationship between variables (Saul *et al.*, 1991).

Fast Fourier transformation was performed to calculate spectral power of the R-R interval, ABP, MSNA, FBV, and TO_m time series, as described previously (Cooke *et al.*, 1999). Briefly, beat-to-beat changes in ABP, MSNA, FBV, and TO_m were linearly interpolated and resampled at 5 Hz to convert the unequally spaced beat-to-beat time series to a uniformly spaced time series for spectral and transfer function analysis. Five minute data sets (1500 samples) were evaluated using a 60-sec window sliding every 10 seconds. These data were detrended, Hanning-filtered, and fast-Fourier transformed to their respective frequency representations. The area under the low frequency (LF, 0.085-0.115 Hz) peak was integrated and averaged for all subjects.

The transfer function (TF) phase, gain, and coherence between neck chamber pressure and ABP, MSNA, FBV, and TO_m was estimated using the cross-spectral method (Saul *et al.*, 1991). The TF analysis reflects the relationship between two measured signals by comparing the amplitude of variability over a specific frequency range. The TF between the two signals was calculated as; $TF = S_{xy}(f) / S_{xx}(f)$, where $S_{xx}(f)$ is the autospectrum of neck chamber pressure and $S_{xy}(f)$ is the cross-spectrum between the autospectrum for neck chamber pressure and the autospectrum for the selected end-organ measurement (Zhang *et al.*, 1998). Transfer function gain values evaluate the relative magnitude between the changes in chamber pressure and the end-organ, while transfer function phase values reflect the relative time relationship between any two measured signals (Saul *et al.*, 1991; Zhang *et al.*, 1998). Using phase delay, the latency from

changes in chamber pressure to each signal was calculated according to the equation; *time delay (sec) = phase (deg) / 360 * frequency (Hz).* The assumption of linearity and reliability of the transfer function estimation was confirmed by coherence (COH) testing of LF spectral power, with coherence values >0.50 considered as confirmation of a significant linear relationship between any two measured variables (Saul *et al.*, 1991).

Statistical Analysis.

Because the power spectrum of some measured signals exhibited a skewed distribution, natural logarithm (ln) transformation was performed before statistical testing, as described previously (Bernardi *et al.*, 1997; Cevese *et al.*, 2001). The effect of sinusoidal NP was evaluated after data was natural log transformed, resulting in an estimate of spectral power based on the raw signal variability. For the absolute power spectral values less than 1, ln transformation produced a negative value. Since exercise produced an increase in signal variability and thus a new baseline, comparisons were made between baseline and sinusoidal NP at rest and during exercise separately. A paired student *t*-test was performed to test for a significant difference between baseline and sinusoidal NP conditions for LF power of HR, ABP, MSNA, FBF, and TO_m at rest and during exercise. Coherence (COH) function in the LF (0.085-0.115) was used as a statistical test to confirm significant linearity between measurements for transfer function gain and phase analysis, with coherence values ≥0.50 considered significant (as described above). All results are expressed as mean ± standard error and statistical significance was set at $P < 0.05$.

RESULTS

Baseline cardiovascular measurements.

During the resting condition, sinusoidal NP significantly increased HR (60.6±2.1 vs. 66.9±2.3 bpm), MAP (90.5±2.1 vs. 100.9±2.8 mmHg), FBV (6.3±0.5 vs. 7.6±0.5 cm/s), and TO_m (0.102±.03 vs. 0.176±0.05 AU) with no significant change in MSNA burst frequency. Exercise significantly increased baseline HR (from 60.6±2.1 to 73.4±3.1 bpm), MAP (90.5±2.1 to 103.9±2.9 mmHg), and FBV (6.3±0.5 to 22.7±1.1 cm/s), with no significant change in MSNA. Absolute TO_m did not significantly change in the exercising or non-exercising leg. During exercise, sinusoidal NP significantly increased HR (73.4±3.1 vs. 77.3±2.7 bpm) and MAP (104.9±2.3 vs. 109.9±2.1 mmHg), with no significant change in FBV or MSNA burst frequency. Exercising sinusoidal NP did not significantly change TO_m of the exercising leg, but significantly decreased TO_m in the non-exercising leg (0.21±0.06 to 0.15±0.06 AU).

Frequency-domain spectral analysis.

Spectral analysis was performed to identify changes in the variability of each signal in the LF (0.085-0.115 Hz) band during baseline and 0.1 Hz sinusoidal NP conditions. During sinusoidal NP, the sympathoexcitation due to positive neck chamber pressure evoked an increase in efferent activity as measured by changes in RRI, MSNA, FBV, ABP, and TO_m. Figure 1 presents the visible entrainment of raw signals and the power spectra for one subject during sinusoidal NP at rest and during exercise.

Compared to baseline, application of sinusoidal NP at rest significantly increased LF spectral power of RRI (5.43±0.32 vs. 7.81±0.24 ln ms^2), ABP (-0.17±0.17 vs. 1.81±0.31 ln $mmHg^2$), MSNA (0.36±0.15 vs. 1.91±0.34 ln AU^2, n=6), FBV (-10.8±0.2 vs. -8.6±0.2 ln AU^2), and TO_m (-11.2±0.3 vs. -8.9±0.2 ln AU^2), see figure 2. During exercise, sinusoidal NP also augmented LF spectral power compared to exercising baseline for RRI (5.18±.027 vs. 7.33±0.22 ln ms^2, P), ABP (0.58±0.24 vs. 1.64±0.28 ln $mmHg^2$, P), MSNA (-0.009±0.57 vs. 1.77±0.58 ln AU^2, n=6, P), and FBV (-8.57±0.2 vs. -7.58±0.14 ln AU^2), see figure 3. The change from baseline to sinusoidal NP was similar between rest and exercise for RRI and MSNA, while exercise attenuated the effect of sinusoidal NP on ABP and FBV (figure 4).

Using near-infrared optodes on both the exercising leg (EL) and non-exercising leg (NEL), we were able to distinguish CBR-mediated changes in TO_m in both active and inactive tissue. At rest, TO_m baseline values and changes during sinusoidal NP were similar between EL and NEL. During exercise, sinusoidal NP increased LF spectral power in both EL and NEL TO_m (-9.6±0.2 vs. -8.2±0.2 AU^2 and -10.5±0.4 vs. -8.9±0.3, respectively). However, when the effect of sinusoidal NP between rest and exercise is compared, the degree of CBR-mediated entrainment is significantly reduced in the EL, with no significant change in the NEL (figure 5).

Cross-spectral transfer function analysis

Cross-spectral analysis was performed to identify the relationship between variables and address the temporal aspects of the efferent responses. We performed transfer function

analysis using neck chamber pressure (CP) as it is the only open-loop "input" to the carotid baroreceptors, and thus all transfer function analysis was arranged accordingly; CP-RRI, CP-ABP, CP-MSNA, CP-FBV, and CP-TO_m. During both resting and exercising baseline conditions, LF (0.085-0.115 Hz) coherence values were well below 0.5 for all cross-spectral comparisons. In contrast, during resting and exercising sinusoidal NP, LF COH was >0.50 for all signals, allowing evaluation of transfer function gain and phase. For both rest and exercise, all phase measurements lagged behind CP changes, i.e. all were negative phase shifts. The phase delay for RRI was established after subtraction of one half-period (i.e. subtraction of 180 degrees) to ensure that the RRI preceding CP changes were not used in the calculation, as described previously (Keyl *et al.*, 2000; Keyl *et al.*, 2001).

At rest, the phase latencies for MSNA (-22.7±4.6 degrees or -631±128 ms), RRI (-24.5±6.4 degrees or -679±178 ms) and FBV (-31.2±5.9 degrees or 840±154 ms) were followed by ABP (-78.5±11.2 degrees or -2.39±0.31 s) and TO_m (-113.5±11.3 degrees or -2.67±0.42 s). Exercise caused a significant shift in phase latency for RRI (-24.5±6.4 to -67.1±8.8 degrees, rest vs. exercise), while the phase shift for ABP, MSNA, FBV, and TO_m were similar to rest. Transfer function gain values were similar between rest and exercise for all end-organ measurements.

DISCUSSION

Using a dynamic sympathoexcitatory stimulus, the present study has demonstrated some degree of carotid baroreflex (CBR)-mediated entrainment of all end-organ measurements during dynamic knee-extension exercise. However, spectral analysis of hemodynamic measurements revealed an attenuated CBR entrainment of arterial blood pressure (ABP), femoral blood velocity (FBV), and tissue oxygenation (TO_m) of the exercising muscle, with no attenuation in the resting muscle tissue. Collectively, these measurements have identified a significant and specific attenuation of end-organ responsiveness to CBR-mediated sympathoexcitation in the vasculature of the exercising muscle. These data suggest a shift towards more predominant local control over the exercising muscle vasculature, but without complete abolition of systemic arterial blood pressure control.

While it is well-established that the CBR remains effective during exercise (Bevegard & Shepherd, 1966; DiCarlo & Bishop, 1992; Potts *et al.*, 1993; Fadel *et al.*, 2001a; Keller *et al.*, 2003; Ogoh *et al.*, 2003b), we believe the present study adds significant new insight into reflex cardiovascular control. Using a prolonged, oscillating NP stimuli rather than a static single pulse of NP, we were able to characterize the *dynamic* nature of CBR control during exercise. Furthermore, inclusion of peripheral hemodynamic measurements extends previous findings by evaluating the influence of exercise-induced metabolites on CBR control of skeletal muscle blood flow and tissue oxygenation. Finally, the use of spectral analysis techniques has provided a novel approach for quantifying the magnitude and temporal relationship of CBR control of peripheral hemodynamics.

Most CBR studies in animals and humans provoke static changes in carotid sinus transmural pressure, producing a sigmoid-shaped curve relating R-R interval or ABP to carotid sinus transmural pressure. Using this technique, an upward and rightward resetting of the curve during exercise has been identified, with no change in CBR-ABP gain (DiCarlo & Bishop, 1992; Potts *et al.*, 1993). Others have evaluated the individual end-organ components of the CBR, showing control of RRI (O'Leary & Seamans, 1993), ABP (Strange *et al.*, 1990), and MSNA (Fadel *et al.*, 2001a; Ogoh *et al.*, 2003b) is unchanged from rest to exercise. However, during exercise the carotid baroreceptors are exposed to perpetual fluctuations in ABP, and so must provide adequate changes in efferent activity on a very dynamic, beat-to-beat basis. As such, we presumed that the carotid baroreceptors might respond differently to an oscillating NP stimulus than to a single pulse of static NP. Thus, while some of the current findings are in contrast to studies using static CBR stimuli (pharmacologic or mechanical), we believe that significant additional information regarding the dynamic nature of CBR control during exercise becomes apparent when an extended period of oscillating input is applied and the average response across several minutes are considered.

Spectral analysis of peripheral hemodynamic control.

In the present study, frequency-domain spectral analysis provided a unique means for quantifying the degree of CBR control over each measured variable. We observed a similar degree of entrainment between rest and exercise for the end-organ measurements of RRI and MSNA, indicating preservation of CBR control for these measurements.

However, during exercise the LF spectral power of ABP, FBV, and TO_m were reduced compared to rest, indicating a diminution in CBR-mediated entrainment of these variables. These findings are consistent with the concept of an altered end-organ responsiveness caused by metabolic byproducts emanating from the exercising muscle, i.e. functional sympatholysis.

Many previous studies have used a pharmacologic approach to assess the metabolic influence on the skeletal muscle vasculature by blocking or activating the post-junctional α-adrenergic receptors. Pharmacologic studies evaluating the responsiveness of these receptors are directly relevant to the present study, as the sympathoexcitation from sinusoidal NP is transduced to the vascular smooth muscle via post-junctional α-adrenoreceptors. The exercise intensity used in the present study has been shown to significantly reduce responsiveness of the vascular α-adrenoreceptors in the human leg (Wray *et al.*, 2003 in press). Attenuation of α-adrenoreceptor responsiveness has also been reported from studies using the selective exogenous agonists in the rat (Anderson & Faber, 1991; Thomas *et al.*, 1994), dog hind limb (Buckwalter *et al.*, 2001), and human forearm (Rosenmeier *et al.*, 2003) during exercise. Data from the present study extend these observations of attenuated end-organ responsiveness to a sympathoexcitatory stimulus, but do so through the distinct use of a reflex "endogenous" CBR-mediated sympathoexcitation, which provides a more physiologic stimulus to the vasculature of the exercising muscle. In addition, the current study had the advantage of provoking reflex NE release across several minutes, which may more closely emulate the events that occur naturally during normal exercise conditions.

Studies in animals (Collins *et al.*, 2001) and humans (Keller *et al.*, 2003) have also utilized reflex maneuvers to evaluate CBR control of skeletal muscle blood flow, with differing results. Using single bouts of NP and NS, Keller *et al.* (2003) identified attenuation of CBR-mediated changes in vascular conductance during unilateral knee extension exercise. In dogs, Collins *et al.* (2001) evaluated the change in vascular conductance of the hind limb and renal vascular bed following carotid sinus hypotension at rest and during ramped treadmill exercise. They reported an increased CBR-mediated change in vascular conductance in the exercising hind limb compared to rest and the renal vascular bed, confirming the importance of the active muscle vasculature in the maintenance of systemic arterial blood pressure during exercise hyperemia. Data from the present study extend these findings with the additional measurements of MSNA and TO_m, providing a comprehensive assessment of CBR control, ranging from reflex autonomic effects to changes at the level of the skeletal muscle microcirculation.

Surprisingly, CBR entrainment of ABP was reduced during exercise, which appears inconsistent with previous findings of a preserved CBR gain from rest to exercise (Bevegard & Shepherd, 1966; Potts *et al.*, 1993). This disparity may be explained by considering differences in the technique of CBR perturbation and in the methods of data analysis. Previous studies using the variable pressure neck chamber have used a static, single NP applied for five seconds and measured the peak change in HR and ABP. Using this static stimulus at several different degrees of neck chamber pressure, the full range of the CBR response has been characterized by a sigmoid-shaped curved using a logistic fit model (Kent *et al.*, 1972). In contrast, the present study utilized an oscillatory NP

technique that alters carotid sinus transmural pressure in a dynamic manner, so that both the "on" and "off" response to the CBR unloading is measured. This approach extends beyond the acute response to a single pulse of NP, characterizing the degree of CBR control across several minutes, and thus providing a more averaged ABP response to alternating changes in carotid sinus transmural pressure.

Importantly, while the degree of CBR-mediated entrainment of ABP, FBV, and TO_m was reduced during exercise, a discreet spectral peak was nonetheless evident for all measurements. This observed preservation of some degree of peripheral hemodynamic control is consistent with the traditional definition of sympatholysis, which suggests a reduced but not ablated responsiveness of the active skeletal muscle vasculature to sympathoexcitation (Remensnyder, 1962; Buckwalter & Clifford, 2001; Joyner & Thomas, 2003). This is a significant observation when considering the functional importance of local blood flow control, since any change in vascular conductance will alter systemic ABP more profoundly as blood flow increases, in accordance with Ohm's law *(mean arterial pressure = cardiac output / total vascular conductance)*. As such, the observed reduction in CBR-mediated entrainment of ABP, FBV and TO_m does not necessarily have a negative impact on cardiovascular homeostasis during exercise. On the contrary, the attenuation in end-organ responsiveness may serve to prevent robust CBR-mediated vasoconstriction in skeletal muscle blood flow that could jeopardize oxygen delivery to the tissue. Thus, the observed attenuation in CBR control at the end organ may in fact serve a protective role, modulating sympathetic input to ensure adequate tissue perfusion while simultaneously supporting ABP during exercise.

Cross-spectral analysis of CBR-mediated sympathoexcitation.

In conjunction with the spectral power for each measurement, cross-spectral comparison of each measurement against the chamber pressure (CP) "input" has provided insight into temporal aspects of CBR efferent activity to the various end-organs. For all measurements, LF coherence values were well above the *a priori* significance criteria of 0.5, indicating a stable relationship between input and output. Transfer function gain (TFG) provided a quantitative ratio of changes in neck chamber pressure and all end-organ measurements in the LF (0.085-0.115 Hz), which remained coherent both at rest and during exercise.

Phase analysis provided an index of latency from the onset of NP to the corresponding response of all end-organ measurements. Previous studies have utilized direct sinus nerve stimulation (Borst & Karemaker, 1983), static NP and NS (Fagius & Wallin, 1980; Wallin & Eckberg, 1982), mathematical modeling (deBoer *et al.*, 1987) and more recently oscillatory NS (Keyl *et al.*, 2001) to evaluate baroreflex latency periods. Collectively, these studies have demonstrated a short delay for RRI (≈500ms) with a greater latency for MSNA from the peroneal nerve (around 1-sec). At rest, we report a similar latency for RRI and a somewhat shorter MSNA latency (631 ms) than previously reported, which may be attributed to methodological differences (i.e. direct recordings versus spectral analysis).

While these cardiovascular latencies have been addressed previously, temporal aspects of CBR-mediated changes in the peripheral blood flow response have not been investigated. In the present study, we observed a progressively increasing phase delay as

CBR-mediated efferent activity moves from the carotid sinus to the peripheral vasculature at rest. Phase latency of MSNA, ABP, FBV and TO_m did not change significantly from rest to exercise, supporting the concept of intact CBR signaling to these end-organs during exercise. Compared to resting values, exercise produced a significant increase in RRI latency in response to the sinusoidal NP. This shift does not reflect an alteration in signaling, but instead is likely due to a shift from fast-acting vagal withdrawl at rest to a more sympathetically-mediated increase in RRI during exercise. To our knowledge, this is the first comprehensive report of latencies from the carotid baroreceptors to the skeletal muscle microcirculation during exercise in humans.

Potential limitations.

One of the potential limitations of the present study regards the use of near-infrared spectroscopy as an index of microcirculatory blood flow. NIRS does not directly measure tissue blood flow, but instead provides a qualitative index of tissue oxygenation. However, recent studies have demonstrated a close correlation between blood flow values measured by plethysmography, the Fick method, and NIRS (Edwards *et al.*, 1993; Van Beekvelt *et al.*, 2001). In addition, validation studies have reported a high correlation between NIRS and conventional ultrasound Doppler measurements in rat hind limb (Fadel *et al.*, 2002) and human forearm (Keller, 2003). Together, these studies support the use of TO_m as an indirect index of microcirculatory blood flow in the present study.

The relatively light exercise intensity used in the present study was necessary to minimize movement artifact that would prevent continuous MSNA recording in the non-

exercising leg, and thus caused only a modest increase in HR with no increase in MSNA burst frequency, though blood flow increased approximately 4-fold to the exercising leg. In a recent study we have identified profound reduction in vascular responsiveness during very light exercise (Wray *et al.* 2003, in press), while others have observed attenuated vascular responses even during the first few contractions of an exercise bout (DeLorey *et al.*, 2002). These previous findings led us to conclude that the exercise intensity we employed increased metabolism to an extent that would allow adequate evaluation of the influence of metabolic factors on CBR-mediated control of the peripheral circulation.

CONCLUSION

Using a dynamic sympathoexcitatory stimulus, the present study has demonstrated CBR control of RRI, ABP, MSNA, FBV, and TO_m during one leg knee-extension exercise. Spectral analysis techniques have identified an attenuated degree of CBR entrainment for all measurements of the peripheral circulation during exercise, suggesting a diminution in end-organ responsiveness. Collectively, these measurements have identified a significant and specific attenuation of end-organ responsiveness to CBR-mediated sympathoexcitation in the vasculature of the exercising muscle. These data suggest a shift towards more predominant local control of the exercising muscle vasculature, but without the loss of control over systemic arterial blood pressure.

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FIGURE LEGEND

FIGURE 1. Sample tracing for R-R interval (RRI), muscle sympathetic nerve activity (MSNA), arterial blood pressure (ABP), femoral blood velocity (FBV), and tissue oxygenation (TO_m) during sinusoidal NP (0.1Hz) at rest (left panel) and during 7W exercise (middle panel). Right panel indicates the corresponding spectra at rest (black fill) and during exercise (gray fill). Note that spectral power of RRI and MSNA are similar between rest and exercise, and are thus superimposed at the LF (0.085-0.115 Hz) frequency range.

FIGURE 2. Resting LF (0.085-0.115 Hz) power of all end-organ measurements during baseline (black bars) and 0.1Hz sinusoidal NP (grey bars) after natural log data transformation. Note that values for FBV are TO_m become negative following natural log transformation since starting value is <1. (*) significantly different than baseline, $P<0.05$.

FIGURE 3. Natural log LF (0.085-0.115 Hz) power of all end-organ measurements during baseline (black bars) and 0.1Hz sinusoidal NP (grey bars) during 7W knee extension exercise. (*) significantly different than baseline, $P<0.05$.

FIGURE 4. Changes in natural log power of all measurements in response to sinusoidal NP at rest (solid grey bars) and during exercise (hatched grey bars). (§) significantly different than resting sinusoidal NP, $P<0.05$.

FIGURE 5. Changes in natural log power of TO_m in the exercising leg and non-exercising leg at rest and during unilateral knee extension exercise. (*) Significantly different than rest, $P<0.05$.

FIGURE 1

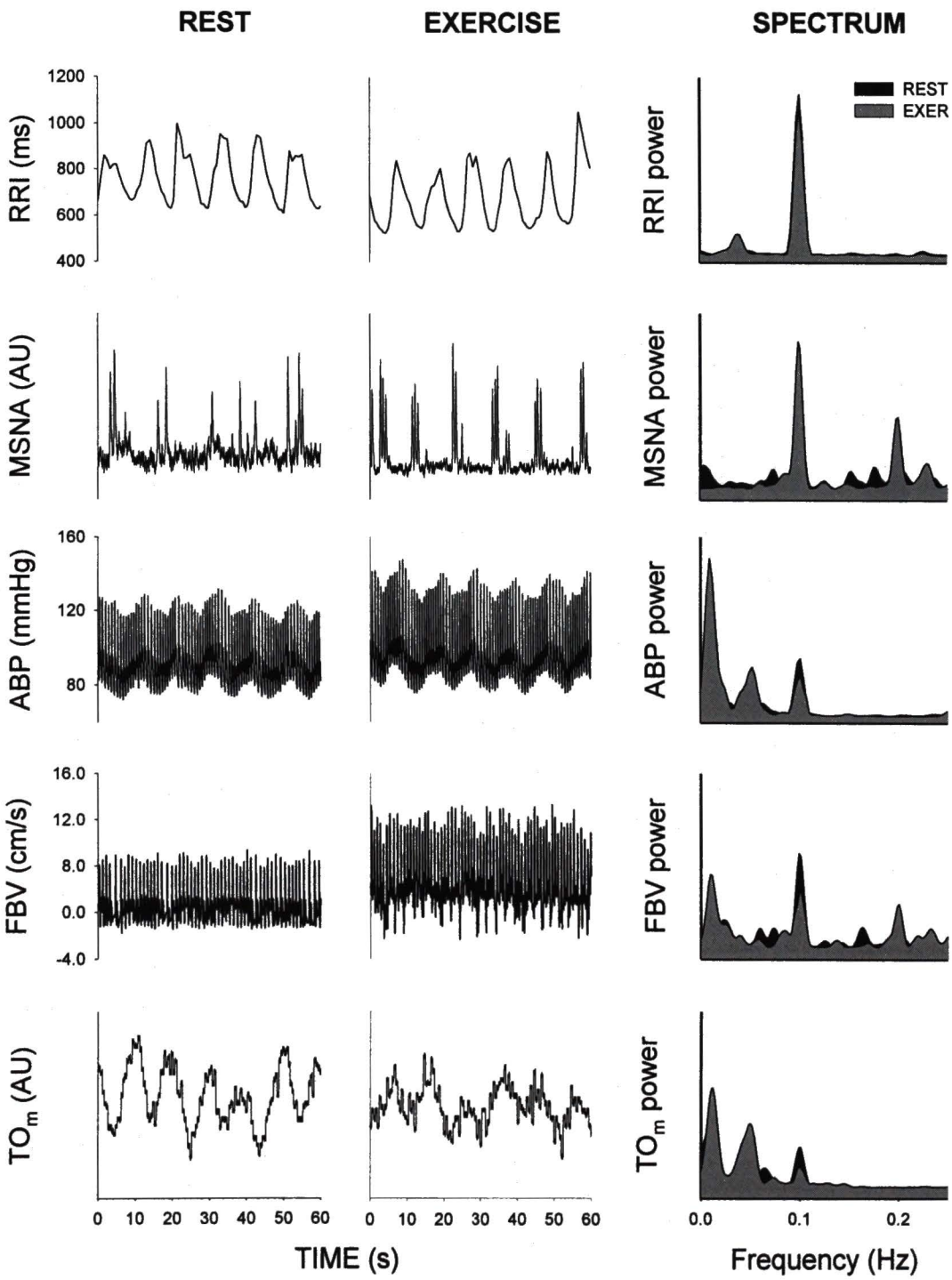

REST
EXERCISE
SPECTRUM
RRI (ms)
1200
1000
800
600
400
RRI power
REST
EXER
MSNA (AU)
MSNA power
ABP (mmHg)
160
120
80
ABP power
FBV (cm/s)
16.0
12.0
8.0
0.0
-4.0
FBV power
TOm (AU)
TOm power
0 10 20 30 40 50 60
TIME (s)
0.0 0.1 0.2
Frequency (Hz)

FIGURE 2

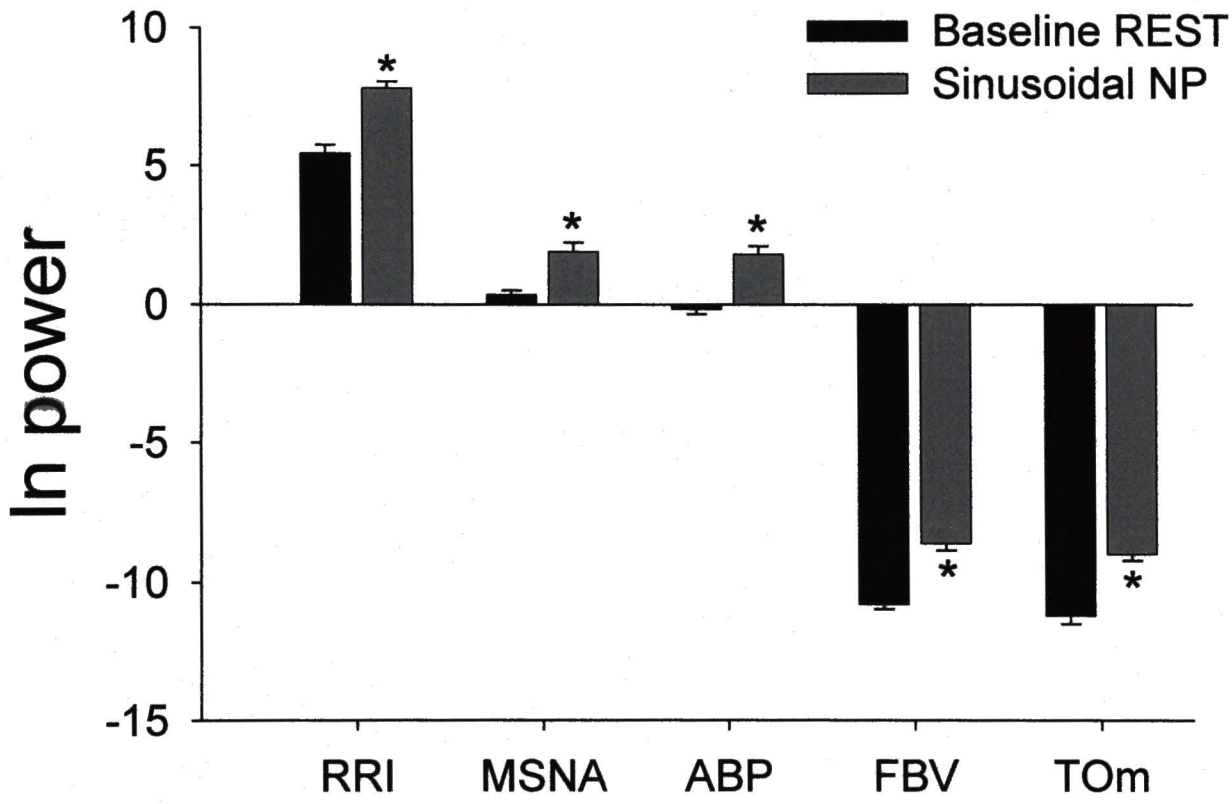

Baseline REST
Sinusoidal NP
ln power
10
5
0
-5
-10
-15
RRI
MSNA
ABP
FBV
TOm
*

FIGURE 3

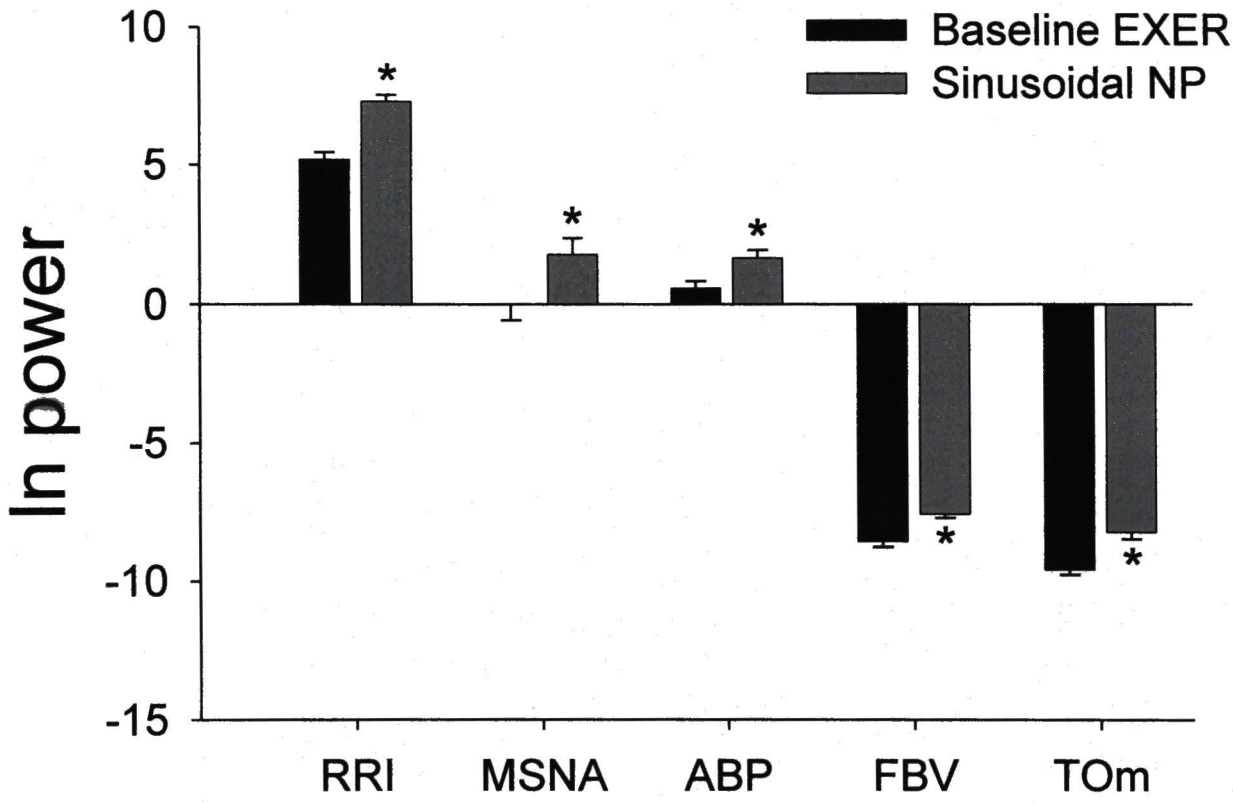

10
5
0
-5
-10
-15
ln power
Baseline EXER
Sinusoidal NP
*
*
*
*
*
RRI
MSNA
ABP
FBV
TOm

FIGURE 4

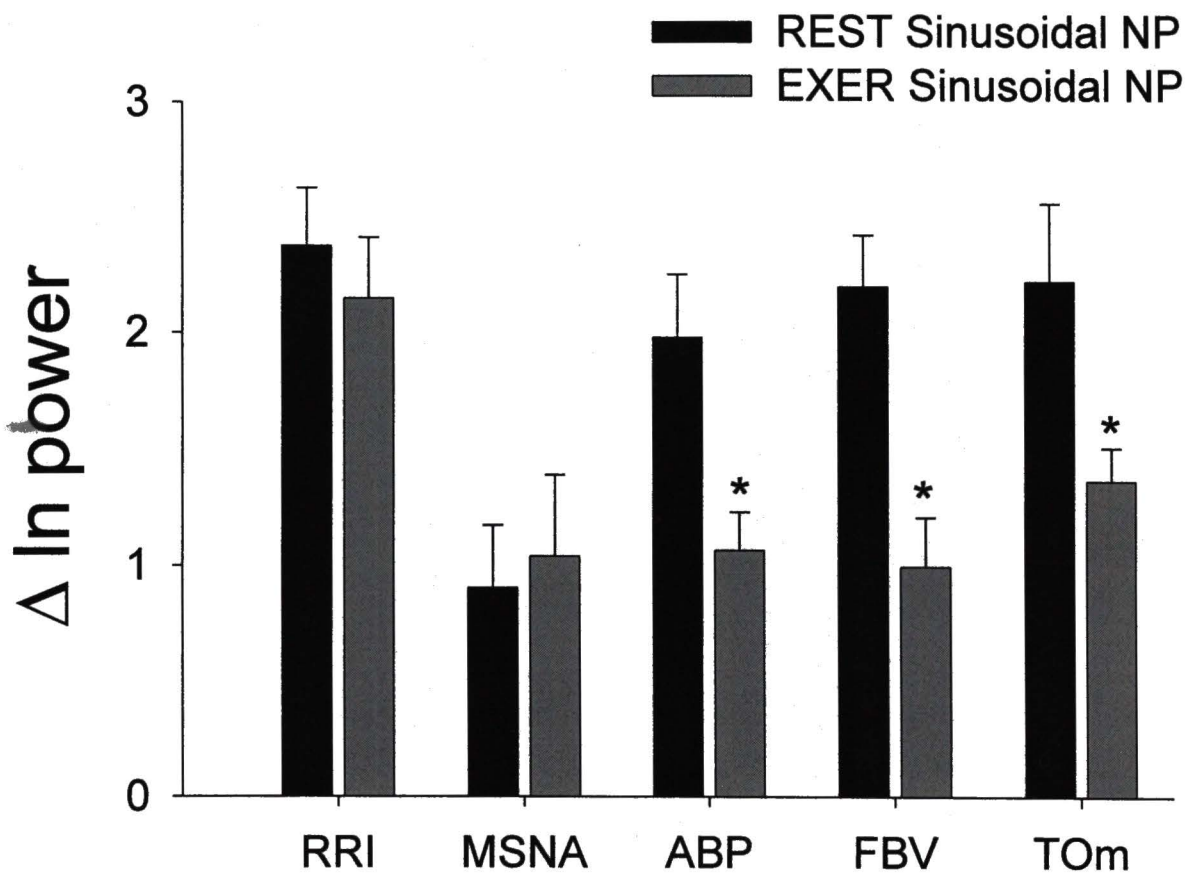
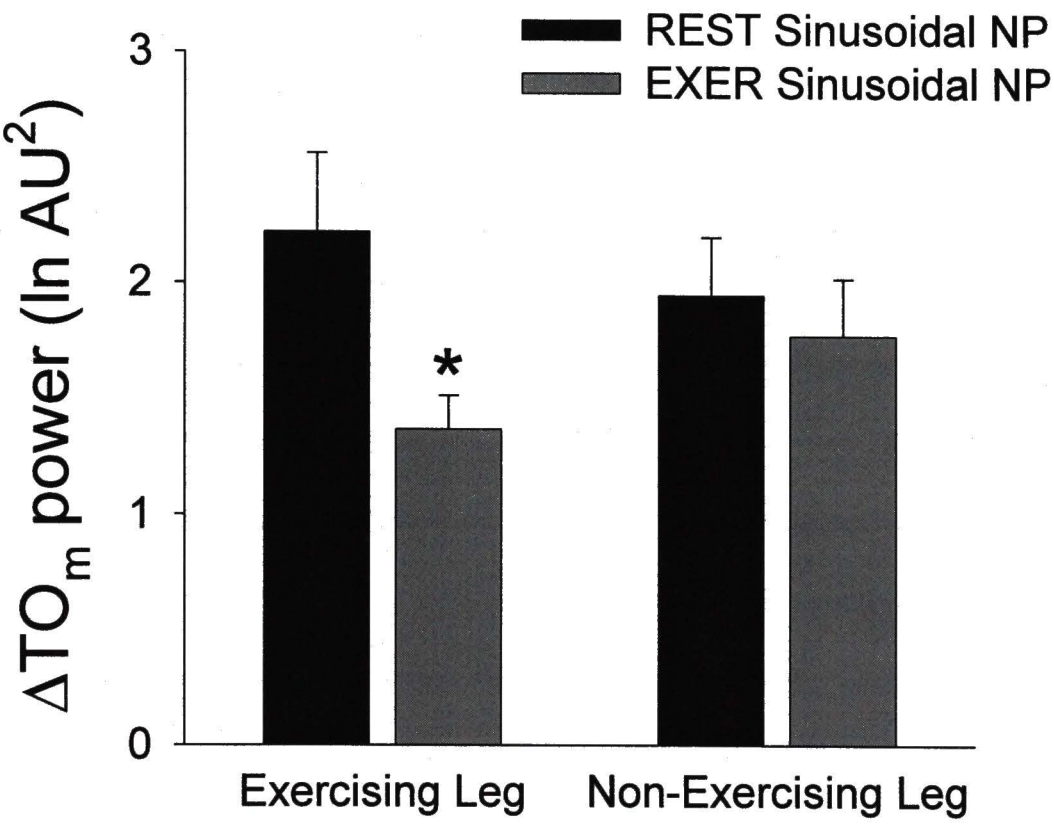

REST Sinusoidal NP
EXER Sinusoidal NP
Δ ln power
3
2
1
0
*
*
*
RRI
MSNA
ABP
FBV
TOm

FIGURE 5


REST Sinusoidal NP
EXER Sinusoidal NP
ΔTO_m power (ln AU^2)
3
2
1
0
*
Exercising Leg
Non-Exercising Leg

CHAPTER V

CONCLUSION

The current project evaluated the interaction of neural and local mechanisms of skeletal muscle blood flow control through *exogenous* (selective agonist drugs) and *endogenous* (CBR-mediated sympathoexcitation) post-junctional α-adrenoreceptor activation. Through selective sympathomimetic drug administration we have identified a functionally differential distribution of α-adrenoreceptor subtypes in the leg skeletal muscle vasculature. Furthermore, we have identified a higher sensitivity of α_2-mediated vasoconstriction compared to α_1 vasoconstriction during exercise, which is dependant on exercise intensity. When oscillatory neck pressure was applied to provoke carotid baroreflex-mediated sympathoexcitation, entrainment of all cardiovascular measurements was seen. However, during exercise CBR control of peripheral hemodynamic measurements were attenuated. Based on these observations, we conclude that exercise attenuates α-adrenergic responsiveness to both exogenous and endogenous activation. This apparent shift in dominance from neural to local control of skeletal muscle blood flow during exercise may serve a protective role, ensuring sufficient muscle blood flow while maintaining systemic ABP homeostasis.

CHAPTER VI

FUTURE DIRECTIONS

Data from the current studies have raised a series of compelling questions suitable for future research efforts. While the current study included selective α-adrenoreceptor agonists to evaluate receptor responsiveness, another interesting approach would involve a similar protocol using selective α-receptor blockade. However, in the human leg even local α-adrenoreceptor blockade produces significant hypotensive effects, making this approach technically difficult. In addition, while we have observed a diminution in α-adrenoreceptor responsiveness with exercise, the impact of exercise-induced metabolites on other endogenous vasoconstrictor substances such as vasopressin and angiotensin II have not been evaluated in humans. These and other putative regulatory pathways must be investigated before a comprehensive understanding of functional sympatholysis may be achieved. The ongoing interest in physiological processes that differ according to gender and age also present potential avenues for future research.

Information regarding the interaction of neural and local mechanisms of skeletal muscle blood flow control may have direct relevance in the clinical setting. Patients afflicted with diseases such as congestive heart failure and type II diabetes frequently experience peripheral vascular disorders, with an associated loss of α-adrenoreceptor responsiveness. This complication may severely limit the normal sympatholytic events

that occurs during muscle contraction to augment muscle blood flow, so that these patients are unable to sustain any challenging exercise for more than a few minutes. Since this patient population may benefit from exercise as part of a rehabilitative treatment program, future studies should consider the pathology that leads to altered α-adrenoreceptor function in these individuals.

CHAPTER VII

INCLUSIVE BIBLIOGRAPHY

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