THE LOCAL CONTROL OF BLOOD FLOW IN THE RESTING AND EXERCISING HUMAN FOREARM

by

Michael Emmerich Tschakovsky

A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of
Doctor of Philosophy
in Kinesiology

Waterloo, Ontario, Canada, 1998

The University of Waterloo requires the signatures of all persons using or photocopying this thesis. Please sign below, and give address and date.

Abstract:

Muscle blood flow at rest and during exercise is determined by the upstream - downstream pressure gradient and the conductance of the vascular bed, which is the result of competing vasoconstrictor and vasodilator influences. The general hypotheses of this thesis focused on determinants of forearm blood flow (FBF) as follows: i) changes in venous pressure contribute to the downstream pressure of the vascular bed and can therefore impact FBF and ii) increases in sympathetic nervous activity (SNA, vasoconstrictor influence) impair the adaptation of FBF to exercise. Four studies were conducted in which beat by beat measures of Doppler ultrasound were used to assess the nature of changes in FBF at rest and exercise in response to alterations in venous pressure and vasoconstrictor influences. In the first study, the role of the muscle pump mechanism in elevating FBF at the onset of exercise was examined. It was observed that while mechanical venous emptying did elevate FBF, it could not account for all of the elevation in FBF following a voluntary contraction, indicating that a rapid vasodilation occurred which was detectable within 2 s of contraction. In the second study, elevating and lowering a resting and exercising forearm above and below heart level revealed a transient vasodilation upon arm elevation possibly mediated by the veno-arteriolar reflex, and a hyperemia upon lowering of the arm. The hyperemia appeared to be the result of both vasodilation induced by arm elevation and venous emptying and was reduced the longer the arm remained above heart level. In the third study, lower body negative pressure was used to elevate SNA. The initial rapid increase in FBF at the onset of forearm exercise was attenuated. However, FBF quickly adjusted during the second phase of adaptation to match that during the control condition, but by 5 min of exercise was again significantly less than control. However, O_2 extraction compensated for the reduced O_2

delivery such that forearm O_2 consumption was maintained. In the fourth study SNA was elevated by calf exercise during calf circulatory occlusion. Forearm vasoconstriction was observed at rest. When exercise began this was quickly abolished and blood flow was elevated in the forearm in proportion to the blood pressure elevations induced by the elevated SNA. It is concluded that venous pressure reductions appear to play a role in determining MBF as hypothesized, by affecting both the downstream pressure and the vascular conductance. However, the hypothesis that elevated sympathetic activity compromises the blood flow adaptation to small muscle mass exercise was not supported by the data. Instead, the evidence from these studies supports the existence of a functional sympatholysis during small muscle mass exercise.

Acknowledgments

First and foremost, I would like to thank my supervisor Rich Hughson. Your encouragement and confidence in me have been instrumental in my development as both a scientist and a person. You saw the value in providing me with challenging situations, and you always made me feel that I could excel. It has been a real blessing to be part of your laboratory "family" and it will provide a model for me to follow when I eventually fill the role of a graduate student supervisor.

Thanks to the members of my committee, Dr. J. Thomson and Dr. M. Sharratt for the many interesting and challenging discussions on physiology in classes, during comprehensives and during this thesis work. Special thanks to Dr. M. Sharratt for his support in me as a teacher of physiology. Teaching was my original motivation for this degree, and your support and encouragement has been very important.

Thanks to the "lab people". Kevin, for being such a fantastic person with whom conversations and ideas flowed. Maureen for taking care of me! From the moment we first met, you've been a wonderful friend and will always be in my heart. To Heather N., Mike C., Rob B., Jorge S., with whom I have shared great friendships. To Dave Northey, a great friend, know that your example of how to deal with work and people in our lab has had a great influence on me.

Thanks to you, Mom and Dad. Each of you have always supported my every choice and your constant reminders to me that you are proud of my path in life have been very important.

Finally, thanks to Caroline. You have seen all sides of me during this learning period in my life, and have accepted all of them without question. How could I go anywhere in life without you?

List of Papers

The following papers form the basis of this thesis. Their chapter designation will be used when referring to them throughout the thesis.

Chapter II: Paper I Vasodilation and muscle pump contribution to immediate exercise hyperemia

Chapter III: Paper II Reductions in venous volume and pressure with passive arm elevation: evidence for a venous volume and pressure contribution to local blood flow

Chapter IV: Paper III -60 mmHg lower body negative pressure induced increases in sympathetic nervous activity: impact on the adaptation of blood flow to exercise

Chapter V: Paper IV Ischemic muscle chemoreflex response elevates blood flow in non-ischemic exercising muscle

Table of Contents

List of Tables

List of Illustrations

List of Abbreviations

(with units where appropriate)

$\dot{Q}a$ - arterial blood flow (ml/min, ml/100ml/min)

FBF - forearm blood flow (ml/min, ml/100ml/min)

MBV - mean blood velocity (cm/s)

MAP - mean arterial pressure (mmHg)

SBP - systolic blood pressure (mmHg)

DBP - diastolic blood pressure (mmHg)

PP - arterial pulse pressure (mmHg)

ΔP - arterial-venous pressure gradient (mmHg)

VC - vascular conductance (ml/min/mmHg)

P_{crit} - critical closing pressure of precapillary vessels (mmHg)

NE - norepinephrine

SNA - sympathetic nervous activity

MSNA - muscle sympathetic nervous activity

LBNP - lower body negative pressure

VOSGP - venous occlusion strain gauge plethysmography

$\dot{V}O_2$ - rate of oxygen uptake (ml/min)

a-vDO_2 - arterial - venous oxygen content difference (mlO_2/100ml blood)

Hb - Hemoglobin

CHAPTER I

Introduction

The flow of blood through a muscle vascular bed provides a means of delivery of important nutrients and removal of metabolic waste products required to sustain muscle metabolism. Muscle cells are capable of rapid and enormous increases in metabolic rate with contractions (Hochachka and Matheson, 1992). This necessitates that blood flow be able to respond rapidly and reach an adequate level in order for muscle performance to be optimal. Both the rate of adaptation (Hughson *et al.*, 1997) and the steady state level (Hogan *et al.*, 1998; van Leeuwen *et al.*, 1992) of blood flow have been shown to impact on muscle metabolism and performance.

Characteristically, the blood flow adaptation in going from rest to exercise is biphasic (Shoemaker *et al.*, 1998; Shoemaker *et al.*, 1996; Shoemaker *et al.*, 1994) (Figure 1.1), with an initial rapid adaptation followed by a second slower adaptation to a steady state level required to meet the new metabolic demands of the muscle. As early as 1935, Anrep and von Saalfeld identified that there was a vasodilatory substance released with the first contraction of exercise which they suggested caused a rapid vasodilation and contributed to an immediate increase in blood flow at exercise onset. However, subsequent research has failed to identify a vasodilatory mechanism that could act rapidly enough to explain the immediate exercise hyperemia. This led others to investigate the role of the muscle pump in elevating blood flow at the onset of exercise (Sheriff *et al.*, 1993). Based on their experiments on dogs running on a treadmill, these investigators suggested that the immediate hyperemia of exercise could be accounted for entirely by the effect of the muscle pump on the local arterial-venous pressure gradient due to contraction-

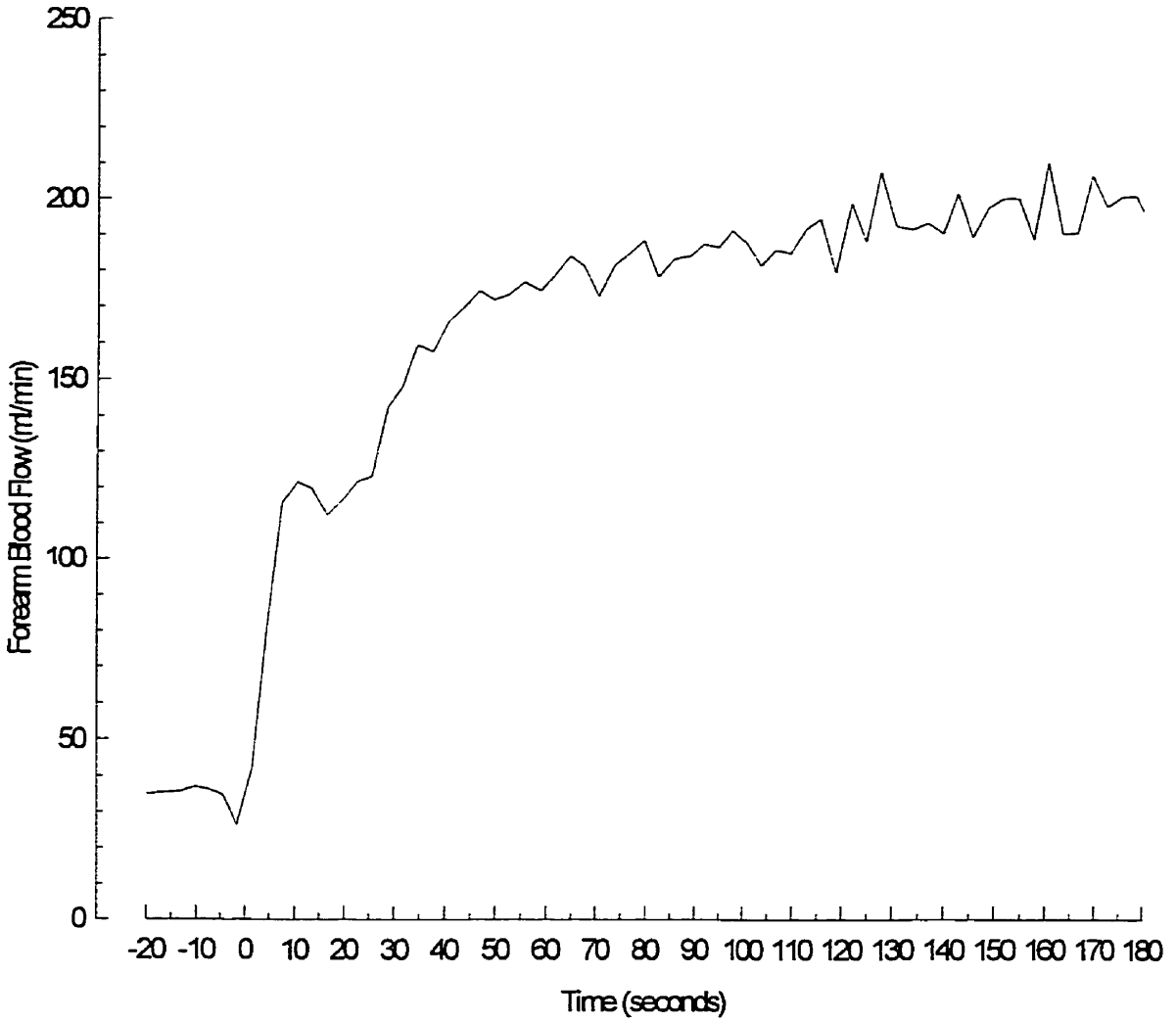

Figure 1.1 *Average blood flow response (9 subjects) during a transition from rest to forearm exercise (8 kg weight, 1 s contraction / 2 s relaxation duty cycle). Exercise began at time = 0 s. The initial rapid increase in forearm blood flow reaches a plateau in 5-10 s. A second, slower adaptation to a steady state level can be seen here beginning ~20 s into exercise in which blood flow adapts to the steady state exercise level. The relative magnitude of the initial fast phase is reduced when a limb exercises above heart level compared to below.*

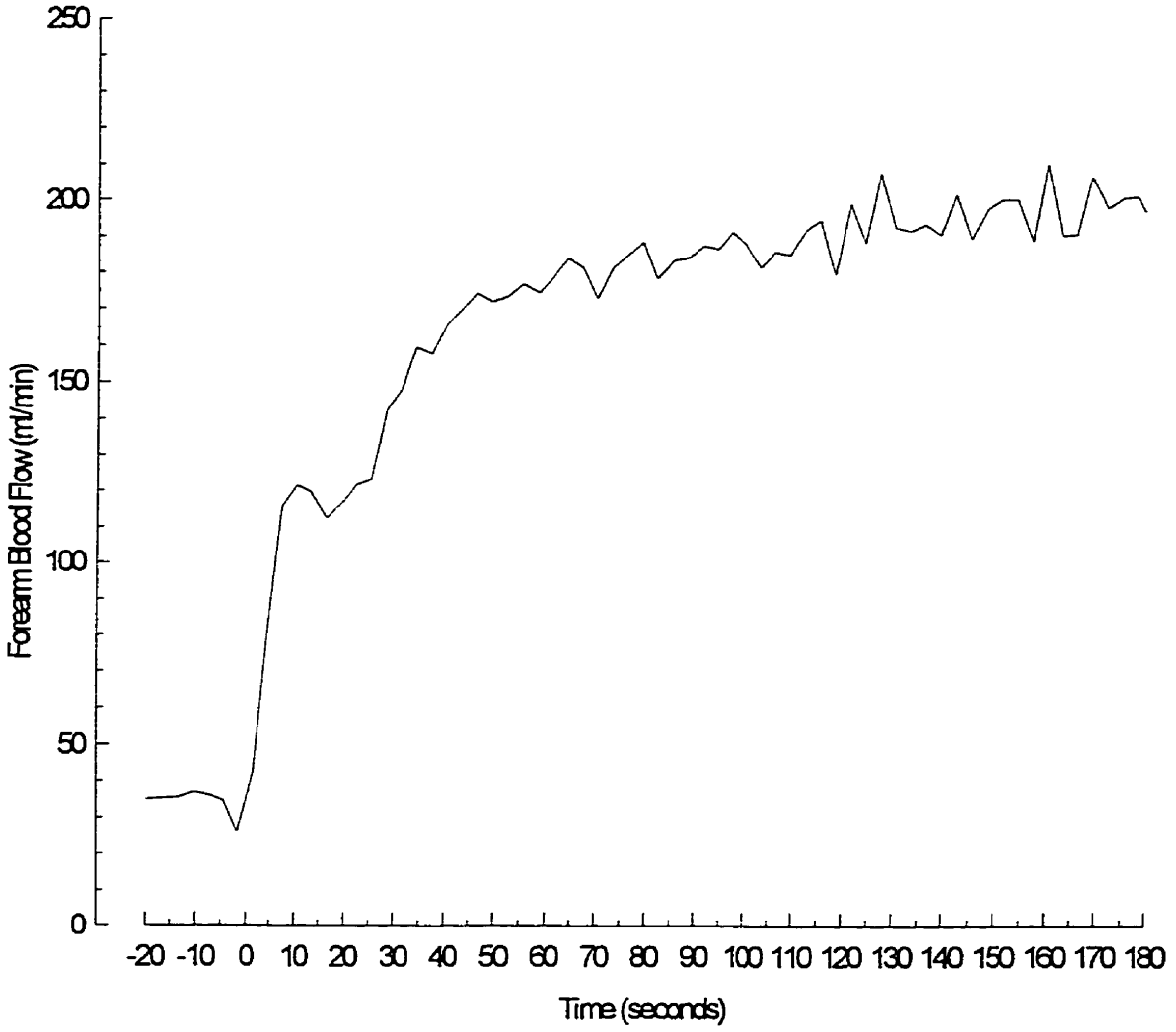

Figure 1.1 *Average blood flow response (9 subjects) during a transition from rest to forearm exercise (8 kg weight, 1 s contraction / 2 s relaxation duty cycle). Exercise began at time = 0 s. The initial rapid increase in forearm blood flow reaches a plateau in 5-10 s. A second, slower adaptation to a steady state level can be seen here beginning ~20 s into exercise in which blood flow adapts to the steady state exercise level. The relative magnitude of the initial fast phase is reduced when a limb exercises above heart level compared to below.*

induced emptying of the veins. The fact that the blood flow response to exercise can be compromised or enhanced depending on the position of the limb relative to heart level (Leyk *et al.*, 1994; Hughson *et al.*, 1997; van Leeuwen *et al.*, 1992) would seem to suggest that the initial resting venous pressure might be important in determining the blood flow response. However, there are a number of studies which suggest that the flow through compliant and collapsible vessels with tone (arterial resistance vessels) is not determined by venous pressure (Spaan, 1985; Shrier and Magder, 1995; Naamani *et al.*, 1995; Saupe *et al.*, 1995; Permutt and Riley, 1963; Jackman and Green, 1990; Braakman *et al.*, 1990).

While a vasodilatory mechanism capable of resulting in immediate (within 1-5 s) flow increases has yet to be determined, numerous metabolites have been identified which cause vasodilation (Shepherd, 1983). However, their effect on the resistance vessels during exercise can often be overridden by increased adrenergic vasoconstrictor influences (Rowell, 1993; Rowell, 1997; Rowell and O'Leary, 1990), though not always (Buckwalter and Clifford, 1998; Thomas *et al.*, 1994; Hansen *et al.*, 1996; Remensnyder *et al.*, 1962). Adrenergic vasoconstriction is activated by pressure sensing reflexes originating in the heart and the carotid artery and aortic arch (Rowell, 1997) and chemosensitive reflexes originating in exercising muscle (Joyner, 1992). The goal of these reflexes may conflict with that of local vasodilatory factors (i.e. vasoconstriction needed to maintain blood pressure vs. vasodilation needed to maintain local blood flow) and potentially compromise the local blood flow to exercising muscle (Saltin, 1988; Secher *et al.*, 1977).

Given the conflicting evidence concerning the role that both venous pressure and elevated sympathetic adrenergic discharge could play in the determination of the blood flow response to

exercise, it was the goal of this thesis to explore their contribution to the control of resting and exercising blood flow to the human forearm. Traditionally, blood flow through a vascular bed ($\dot{Q}_a$) has been modelled as a form of Ohm's law for the circulation

$$\dot{Q}_a = \Delta P \cdot VC \quad \text{Eqn.1}$$

Factors involved in the control of blood flow in resting or exercising muscle would therefore fall under two general categories: either they influence the effective pressure gradient (ΔP) across the vascular bed or the vascular conductance (VC) of that bed.

Using this model as a framework, there were two major hypotheses in this thesis.

i) it was hypothesized that changes in venous pressure do contribute to the downstream pressure of the vascular bed and can therefore impact on muscle blood flow

ii) it was hypothesized that increases in sympathetic nervous activity (SNA, vasoconstrictor influence) impair the adaptation of muscle blood flow to exercise.

The following sections will now explore in more detail the issues surrounding i) the role of venous pressure in determining ΔP and ii) the competition between sympathetic vasoconstriction and local vasodilatory influences in determining VC. A reduction in VC due to elevations in sympathetic adrenergic vasoconstrictor activity will be termed a "sympathetic restraint" (Shepherd, 1983). The opposite effect, that of local control factors inhibiting the release of norepinephrine from sympathetic nerve terminals or overriding sympathetic vasoconstriction such that the resistance vessels are less sensitive to adrenergic stimulation will be termed a "functional sympatholysis" (Laughlin *et al.*, 1996).

Venous Pressure Contribution to the Effective ΔP

While it is obvious that arterial pressure constitutes the upstream pressure, it has been

difficult to determine what acts as the effective downstream pressure. Some investigators have provided evidence for venous pressure (Sheriff *et al.*, 1993; Folkow *et al.*, 1971), while the evidence from experiments of others suggest a critical closing pressure (P_{crit}) of the arterioles which acts as a Starling resistor (Shrier and Magder, 1995; Naamani *et al.*, 1995; Magder, 1990) a concept first developed by Permutt and Riley (1963). Finally, others have provided evidence that the effective downstream pressure determining arterial inflow is in a compliant region of the arteriolar circulation (Spaan, 1985; Saupe *et al.*, 1995).

Venous Pressure and the Muscle Pump

A major focus of this debate as it relates to the blood flow response to exercise has revolved around the concept of the muscle pump (Magder, 1995; Laughlin, 1987). Maximal blood flow during muscle contractions is greater than the flow achieved when maximal vasodilation is induced by pharmacological intervention (Laughlin, 1987). This suggests that there is an additional contribution of muscle contractions in determining blood flow. In human studies it has been observed that blood flow is higher during intense exercise when the limb is below vs. above heart level (van Leeuwen *et al.*, 1992; Leyk *et al.*, 1994; Folkow *et al.*, 1971) and that it adapts more rapidly (Hughson *et al.*, 1997; Leyk *et al.*, 1994). Such an effect on exercising muscle perfusion has positive consequences on muscle metabolism and performance (Hogan *et al.*, 1998; van Leeuwen *et al.*, 1992; Hughson *et al.*, 1997), therefore the mechanism responsible is of considerable interest. The reason for the improved blood flow during muscle contractions in the dependent position might be explained by a contraction induced improvement in the local arterial-venous pressure gradient (Folkow *et al.*, 1971; Sheriff *et al.*, 1993). Briefly, position of a limb below heart level increases both local arterial and venous pressures equally

through a hydrostatic effect. Muscle contraction squeezes blood through the veins toward the heart and venous valves prevent back flow (Pollack and Wood, 1949; Barendsen and van den Berg, 1984; Stegall, 1966; Folkow *et al.*, 1971) such that venous pressure is lowered, improving the arterial-venous pressure gradient. Additionally, it has been suggested that pressure in the venules might actually become negative upon muscle relaxation if they were tethered to the surrounding tissue (Laughlin, 1987), further contributing to a widening of ΔP, although the duration of such negative pressure would have to be brief. What also appears evident is that the type of muscle (deep as opposed to superficial) and the type of contraction (voluntary, dynamic contractions vs. tetanic stimulation) influences the magnitude of the muscle pump contribution to exercise hyperemia (Terjung and Mackie Engbretson, 1988; Laughlin, 1987). These differences could be explained by the efficiency of venous emptying achieved by different muscles and types of contractions. For example, deeper muscles might be expected to achieve greater intramuscular pressures, possibly improving venous emptying. Dynamic contractions, where fibers contract asynchronously, might be expected to squeeze more blood towards the heart than tetanic contractions where all the fibers contract simultaneously. In both cases the added reduction in average venous pressure would then result in a greater arterial-venous pressure gradient, facilitating muscle blood flow.

It is important to provide a cautionary point when describing the circulation as a version of Ohm's law within the context of a muscle pump mechanism. Because the local pressure gradient cannot be measured, the net effect of the muscle pump on flow would appear as an increase in vascular conductance as traditionally calculated, where $VC = \dot{Q}_x/\Delta P$, and arterial pressure (assuming a venous pressure close to 0 in the right atrium) represents ΔP. Therefore,

the term "virtual" conductance has been coined (Sheriff *et al.*, 1993) for such calculations to indicate that a component of the observed increase in flow for a given arterial driving pressure could in part be due to the potential energy imparted by the expulsion of venous volume (increase in the local pressure gradient) and the kinetic energy imparted by the pumping action of the muscle (Sheriff and Van Bibber, 1998). At the same time that flow might be enhanced between contractions, the compression of the blood vessels during contraction severely limits flow (Shoemaker *et al.*, 1998; Robergs *et al.*, 1997; Kagaya and Ogita, 1992; Walloe and Wesche, 1987; Anrep and von Saalfeld, 1935). Beat-by-beat observations of blood flow during rhythmic contractions indicate that blood flow is minimal during the time that the muscle is contracted and occurs predominantly during the relaxation phase (Robergs *et al.*, 1997; Kagaya and Ogita, 1992; Walloe and Wesche, 1987). Given the energy that is therefore imparted into the system, both in terms of limiting and enhancing blood flow, it has been rightly suggested that the use of Ohm's law as a representation of local hemodynamics would be incorrect "across a vascular circuit that is broken up by a pump that adds energy" (Laughlin, 1987). But this would only be the case if flow was averaged over the course of muscle contraction/relaxation cycles. The basic tenet of Ohm's law should hold for the flow occurring between contractions, since this would depend only on the true vascular conductance and the local pressure gradient. However, since the true local venous pressure cannot be measured, the expression of ΔP within equation 1 is based on arterial pressure only and would not change. Therefore, the effect of changes in local venous pressure would appear as changes in VC in equation 1.

Venous Pressure: Vascular Waterfall and Arterial Compliance Models

Not all evidence points to a contraction induced reduction in venous pressure augmenting

muscle blood flow. Studies using isolated animal muscle preparations (Jackman and Green, 1990; Braakman *et al.*, 1990; Permutt and Riley, 1963; Magder, 1990; Shrier and Magder, 1995; Naamani *et al.*, 1995) indicate that vessels with tone create an effective back pressure, also known as a critical closing pressure (P_{crit}) (Shrier and Magder, 1995), at some point upstream of the capillaries such that venous pressure below this effective back pressure does not affect arterial inflow. This has been termed the 'vascular waterfall' phenomenon (Permutt and Riley, 1963) in that arterial inflow behaves like flow down a river above a waterfall. Here, the Starling resistor effect of the tone in the resistance vessels at some point upstream of the capillaries is the point of the waterfall and venous pressure is akin to the level of the water in the pool below the waterfall. The flow down the river is impacted by the resistance and the pressure gradient above the waterfall, but the pool below the waterfall cannot affect flow over the waterfall unless the level of the pool matches that of the waterfall. Likewise, arterial inflow to a vascular bed would be determined by the resistance to flow on the arterial side and the difference between the arterial pressure and P_{crit}. Venous pressure would only affect arterial inflow if it exceeded P_{crit}.

P_{crit} appears to be affected by resistance vessel tone, but is not abolished even at maximal vasodilation in the vascularly isolated, pump-perfused dog hindlimb (Shrier and Magder, 1995). The repeated observations of zero flow through an isolated dog muscle preparation in the presence of an arterial-venous pressure gradient (Shrier and Magder, 1995; Shrier and Magder, 1993; Magder, 1990) which can range from less than 10 mmHg under maximally vasodilated conditions to over 60 mmHg (Shrier and Magder, 1995) constitutes the predominant evidence for a vascular waterfall phenomenon, since the traditional view of flow depending on the arterial-venous pressure gradient would predict that under such conditions there should still be flow.

There are however some arguments against the interpretation of a positive arterial pressure with zero inflow in isolated hindlimb preparations as an indication of a functional vascular waterfall in the in vivo condition. To determine the P_{crit} representing the waterfall in such a preparation, dynamic pressure-flow measurements are taken. Femoral artery inflow and venous outflow are isolated and the femoral artery is channeled through a pump which allows manipulation of hindlimb arterial inflow. Flow and pressure measurements are made at a site in the femoral artery prior to it entering the hindlimb. Pump flow is then progressively reduced to zero over a few seconds. The measured arterial pressure when flow ceases is then assumed to represent the critical closing pressure causing downstream vessel collapse. However, it has been suggested that this zero flow arterial pressure does not represent vessel collapse pressure, but rather the pressure in a compliant vascular capacitance downstream from the point of flow and pressure measurements (Spaan, 1985). The explanation is as follows: when arterial inflow, and therefore pressure, is reduced over a few seconds it should eventually equal pressure in this compliant region and arterial inflow would then stop. However, the compliant region would continue to discharge blood through the capillaries since its downstream pressure is that in the post-capillary venules, therefore actual flow would not stop. This model predicts that, in the experimental preparation just detailed, a progressive reduction in arterial pressure would occur while arterial inflow remained at zero.

To address this controversy, Magder (1990) performed a series of experiments in which he 1) varied the rate at which pump flow was reduced so that the time to zero flow ranged from 1-10 s 2) raised femoral venous outflow pressure in small steps. He predicted that altering the rate of flow reduction would not be expected to affect the measured P_{crit} if a Starling resistor

determined P_{crit}. Likewise, venous pressure increases up to P_{crit} should not reduce arterial inflow. The results indicated 1) that the measured P_{crit} was reduced with increasing time to zero flow and that arterial pressure continued to decrease even after arterial inflow stopped, indicating continued flow from arterioles to veins 2) While P_{crit} was measured at 50-60 mmHg in the resting dog hindlimb, arterial inflow was reduced with venous pressure above ~15 mmHg, while in the vasodilated hindlimb any change in venous pressure affected arterial inflow. These results clearly support the existence of an arterial compliance, and also indicate that venous pressure would be expected to influence arterial inflow under most physiological conditions. There was some indication that a vascular waterfall does exist, namely the observations that 1) arterial pressure once flow ceased did finally plateau at levels above venous pressure 2) elevating venous pressure from 0-15 mmHg did not affect arterial inflow. However the data suggest that the vascular waterfall likely has little impact under most physiological conditions.

Proponents of an arterial compliant region determining the effective back pressure to arterial inflow also suggest that venous pressure does not directly determine arterial inflow in a pulsatile pressure system as occurs in vivo, since arterial inflow during diastole at rest is zero despite a positive arterial-venous pressure gradient. With pulsatile pressure, this region appears to function as a capacitor which effectively "stores" blood volume during systole and ejects it during diastole via elastic recoil (Spaan, 1985). Support for this "windkessel" effect comes from a recent study by Saupe et al. (1995). In arteries feeding resting muscle, there is often no flow during diastole (Saupe *et al.*, 1995; Ehrlich *et al.*, 1980) despite the fact that diastolic pressure is above venous pressure. These investigators reasoned that, if a Starling resistor determined arterial inflow, then the duration of diastole should not affect the flow during the subsequent beat,

because the collapse pressure of precapillary arterioles should remain constant. However, if the effective back pressure to flow was determined by an arteriolar compliance, then an increased diastolic time should allow for a greater discharge of blood volume from that region and a lower pressure by the time the next beat occurs. Consistent with this was their observation of a larger systolic pulse of blood following a longer R-R interval (Saupe *et al.*, 1995).

However, in such a model, the discharge of blood from the arteriolar compliant region occurs against a downstream pressure which is venous pressure. Therefore this venous pressure should determine the rate of discharge of the compliant region. This means that venous pressure determines the degree to which this compliant region is filled and therefore its pressure, which means that in this model venous pressure would be expected to influence arterial inflow into the compliant region.

Venous Pressure and the Veno-Arteriolar Reflex

It is important to note that, while venous pressure changes may or may not have a direct mechanical effect on arterial flow, evidence also exists for a reflex neural communication between veins and arterioles in which changes in venous pressure can alter arterial vascular conductance (Henriksen *et al.*, 1983; Henriksen, 1991; Chen *et al.*, 1995; Vissing *et al.*, 1997; Henriksen and Sejrsen, 1977). This is observed in subcutaneous tissue (Vissing *et al.*, 1997; Nielsen *et al.*, 1988; Skagen, 1982; Henriksen and Sejrsen, 1977; Henriksen, 1991) and muscle (Henriksen, 1991; Henriksen *et al.*, 1983; Haddy and Gilbert, 1956; Henriksen and Sejrsen, 1977). The concept of a reflex vasoconstriction in response to increasing venous volume and pressure was first proposed by Gaskell and Burton (1953). Evidence supporting such a reflex comes from studies in which lowering of a limb into the dependent position has been shown to result in a reflex

vasoconstriction, which can be eliminated by local neural blockade (Henriksen and Sejrsen, 1977; Vissing *et al.*, 1997) but not by spinal sympathetic blockade (Henriksen and Sejrsen, 1977) suggesting a local reflex mechanism. Rygaard et al. (1991) have provided anatomical evidence that sympathetic nerve fiber collaterals from the sympathetic arteriolar plexus innervate concomitant venules in dog skeletal muscle, and that some of these fibers return from the venules to the arterial network. They suggest that this might provide the anatomical link between venules and arterioles through which the local veno-arteriolar reflex is mediated.

Generally, the veno-arteriolar reflex is believed to exhibit threshold behaviour, such that vasoconstriction is initiated when venous pressure rises above ~25 mmHg (Henriksen, 1991). At this point a pronounced vasoconstriction is observed. However, one study observed a progressive decrease in skin blood flow on the dorsum of the hand relative to that measured at heart level which began with the arm as little as 10 cm below heart level (Petersen and Sindrup, 1990). Further decreases in flow were evident as arm position became more dependant. An additional characteristic of the veno-arteriolar reflex mediated vasoconstriction is that it is maintained over time (Henriksen *et al.*, 1983). In fact it appears to be able to, in conjunction with myogenic responses, defend arterial pressure upon assuming upright stance when central sympathetic vasoconstrictor pathways have been blocked (Henriksen *et al.*, 1983).

The veno-arteriolar reflex has most often been investigated in terms of the vasoconstriction induced by venous congestion. However, it would be expected that the removal of venous congestion would therefore release this vasoconstrictor influence, resulting in a vasodilation. Consistent with a vasodilatory response to the reduction of venous pressure in a dependent limb would be the observation of a vasodilation initiated by contraction-induced

emptying of muscle veins (the muscle pump). Indeed, it has been demonstrated that the vasoconstriction observed in skin and resting muscle upon moving the limb into the dependent position is abolished when the muscle pump is activated by muscle contractions (Henriksen and Sejrsen, 1977; Nielsen, 1982; Nielsen *et al.*, 1988). Venous stasis during exercise restores this constriction (Henriksen and Sejrsen, 1977; Nielsen, 1982), suggesting that it is specifically the venous emptying induced by contractions which triggers the arterial vascular response with exercise. The effect during exercise appears to be maintained (Nielsen, 1982).

Vasoconstrictor Influence on the Blood Flow Response in Exercising Muscle

While the effective pressure gradient and venous pressure mediated reflexes might contribute to blood flow at rest and during exercise, the enormous potential of muscle for receiving blood flow in excess of 300 ml/100ml/min (Rowell, 1988) during exercise depends predominantly on the vasodilation of resistance vessels. Local vascular tone can be reduced by a number of vasodilatory factors, some of which are metabolic in nature (e.g. PO_2, La^-, CO_2, P_i, adenosine), flow mediated (e.g. nitric oxide, prostaglandins, acetylcholine), neural (e.g. β-adrenergic) or linked with muscle activation (e.g. K^+, acetylcholine) (Shepherd, 1983). In addition, the reduction of α_1 and α_2 receptor mediated sympathetic vasoconstrictor activity would be expected to have a vasodilatory effect.

Increases in vascular tone can be achieved by increases in direct sympathetic stimulation of α_1 and α_2 receptors on vascular smooth muscle (Laughlin *et al.*, 1996) and the action of circulating agonists (Faber, 1988). The presence and distribution of the adrenergic receptor subtypes varies with the level of the arteriolar tree. α_2 receptors were originally thought to exist only on adrenergic nerve endings or their junction with smooth muscle, but their post-junctional

existence has been confirmed (Drew and Whiting, 1979). α_1 receptors are also found junctionally and post-junctionally (Ohyanagi *et al.*, 1991). Concentration-response curves (vessel diameter changes) for selective α_1 and α_2 agonists in the absence or presence of selective α_1 and α_2 antagonists (Faber, 1988) indicate that adrenergic regulation of large arterioles is dependent on both receptor types, while small terminal arterioles are controlled predominantly by α_2 receptors. This difference in spatial distribution might suggest different roles in vascular control. Within the context of competition between adrenergic vasoconstrictor and metabolic vasodilator influences on arteriolar tone, it has been shown that α_2 mediated vasoconstriction is far more sensitive to metabolic inhibition induced by muscle contraction (Buckwalter and Clifford, 1998; Thomas *et al.*, 1994; Anderson and Faber, 1991), i.e. a functional sympatholysis. Since the terminal arterioles are thought to contribute predominantly to capillary recruitment and not vascular conductance, it has been suggested that such sensitivity means that sympatholytic effects regulate capillary perfusion (Laughlin *et al.*, 1996), but not vascular conductance (Rowell, 1997). However, it has been shown that α_2 mediated functional sympatholysis clearly affects vascular conductance (Thomas *et al.*, 1994) as well.

At rest in skeletal muscle, local sympathetic influences in the presence of minimal vasodilator influences dominate, as evidenced by the typical levels of resting flow in the human forearm of ~2-6 ml/100ml/min (Corcondilas *et al.*, 1964; Strandell and Shepherd, 1967; Williams *et al.*, 1985; Tschakovsky *et al.*, 1996) compared with the maximal capacity for blood flow in excess of 300 ml/100ml/min (Rowell, 1988; Saltin, 1988). Of course, limb blood flow represents a combination of muscle, skin and adipose tissue blood flow. Elia and Kurpad (1993) have partitioned resting forearm blood flow into muscle and skin components and found that muscle

flow ranged from 1.4 - 1.8 ml/100ml/min under conditions where total forearm blood flow was ~50-100% greater than flow to the muscles within them. Their measures of skin blood flow were 9.1 ml/100ml/min. Therefore, it would appear that resting forearm blood flows higher than 1.4-1.8 ml/100ml/min are attributable to the contribution of skin blood flow. Application of LBNP to unload cardiopulmonary receptors (Vissing *et al.*, 1994; Baily *et al.*, 1990; Sundlof and Wallin, 1978) or initiation of a chemoreflex with ischemic exercise (Hansen *et al.*, 1994; Victor *et al.*, 1988) elicits increases in muscle sympathetic nerve activity (MSNA) in resting limbs and have almost without exception resulted in reductions in resting limb vascular conductance (Tripathi and Nadel, 1986; Tripathi *et al.*, 1989; Shoemaker *et al.*, 1997; Strandell and Shepherd, 1967; Hansen *et al.*, 1994; Joyner *et al.*, 1990), showing that vasoconstrictor influence can be increased even further in resting muscle.

Large Muscle Mass Exercise

Most early measures of exercising muscle blood flow in humans were obtained with venous occlusion plethysmography and ^{133}Xe clearance. As summarized by Saltin (1988) and Rowell (1988) these techniques provided peak blood flow values of 50-100 ml/100ml/min in exercise. At that time, a review of the field by Mellander and Johansson (1968) concluded that, with an estimate of 30 kg of muscle working maximally, the required muscle blood flow would be 15-18 L/min, which was within the cardiac pumping capacity of normal subjects (maximal cardiac outputs 20-24 L/min). This meant that maximal vasodilation could occur without a compromise to arterial blood pressure and it would be expected that peak oxygen uptake would be linearly related to exercising muscle mass. However, when arm exercise is added to leg exercise in humans, peak oxygen uptake only increases 2-3 % (Saltin, 1988). This suggests a blood flow

limitation. The question is what might be preventing blood flow from increasing in proportion to exercising muscle mass?

Using thermodilution techniques and a single leg exercise model, Andersen and Saltin (1985) clearly demonstrated that muscle blood flows far in excess of those previously measured could be achieved and provided the impetus for a renewed examination of blood flow control in exercise. Based on the tremendous capacity for blood flow in exercising muscle, Rowell (1988) has drawn attention to the mass of exercising muscle and its relation to maximal cardiac pumping capacity. His analysis, which will be detailed here, provides a framework for understanding the role of sympathetic vasoconstriction in exercising muscle.

Resting tissue blood flow during exercise is approximately 3 L/min. In situations where the total muscle mass (~30 kg in a 75 kg "normal" subject) or the majority of the muscle mass is exercising intensely, the cardiac output required to maintain flows of 200 ml/100ml/min or more to all of the exercising muscle mass would be in excess of ~60L/min. This would outstrip the pumping capacity of the heart by almost 3-fold. Obviously if the muscle resistance vessels were allowed to dilate fully, systemic arterial blood pressure could not be maintained. However, in exercise blood pressure is observed to increase with intensity (Rowell, 1993) and the recruitment of additional muscle mass (Lewis *et al.*, 1983). This is due to a combination of increased cardiac output and increased systemic sympathetic vasoconstrictor activity via baroreflex and chemoreflex mechanisms (Sinoway and Prophet, 1990; Kaufman and Forster, 1996). While initial elevations in blood pressure may be achieved by constriction in resting tissue (Rowell, 1993), in order for this elevation in blood pressure to occur as muscle recruitment nears levels which could outstrip the pumping capacity of the heart the exercising muscles must eventually become the target of

sympathetic vasoconstriction. Therefore it has been suggested that elevated levels of sympathetic activity with increasing exercise intensity can partially override local vasodilatory signals and act to restrain active muscle blood flow (a "sympathetic restraint") in order to avoid outstripping the pumping capacity of the heart (Secher *et al.*, 1977; Saltin, 1988; Laughlin *et al.*, 1996). The opposite effect, whereby vasodilatory influences overwhelm elevated sympathetic discharge is termed a functional sympatholysis (Shoemaker *et al.*, 1997; Saltin, 1988). The conclusion that a sympathetic restraint of exercising muscle blood flow must occur when cardiac pumping capacity is threatened is inescapable, since hypotension is not observed during even maximal exercise. However, a review of evidence exploring this question to date suggests that sympathetic activity in exercising muscle can be elevated substantially before such a vasoconstriction occurs. This suggests that the interaction of adrenergic vasoconstrictor and metabolic vasodilator influences is complex.

One of the first studies to investigate a sympathetic restraint of exercising muscle blood flow was by Secher and colleagues (1977) in which arm cranking exercise was added to leg cycling exercise. These investigators reported a decrease in leg blood flow upon addition of arm exercise, indicating a leg vasoconstriction (combined arm and leg exercise was equivalent to 77% $VO_{2\ max}$.). However similar subsequent studies by Savard et al. (1989) (71% $VO_{2\ max}$, arm cranking exercise added to leg exercise), Richter et al. (1992) (82% $VO_{2\ max}$, arm cranking exercise added to leg exercise) and Saito et al. (1992) (rhythmic forearm exercise added to calf plantar flexion exercise) observed that, even though indicators of sympathetic nervous activity to the exercising leg, or the contra lateral resting leg, increased with the addition of arm exercise (assessed by norepinephrine (NE) spillover (Richter *et al.*, 1992; Savard *et al.*, 1989) and micro

electrode measures of muscle sympathetic nervous activity (MSNA) (Saito *et al.*, 1992)), local blood flow did not change. However, since systemic blood pressure was increased with the addition of the arm exercise, it follows that leg vascular resistance did increase in these studies. It may be, as stated by Richter and colleagues (1992), that this increase in leg vascular resistance acted to prevent leg over perfusion.

In attempts to determine the effect at higher relative work intensities, Saltin (1988) reported reductions in leg blood flow at 80-90% $\dot{V}O_{2\,max}$ with NE spillover levels of ~900 ng/min. In contrast, Richardson et al. (1995) compared leg blood flow during a progressive maximal leg kicking exercise test with the same test performed with added progressive arm cranking. Despite a 4-fold higher leg NE spillover in the combined arm and leg exercise (4215 vs. 901 ng/min), these investigators found no reduction in leg blood flow, even at maximal workrates. Taken together the literature suggests that increased sympathetic activity is able to overcome local vasodilatory stimuli and effect an increase in exercising skeletal muscle vascular resistance, but sympathetic activity does not appear to compromise blood flow, at least under the conditions studied.

Small Muscle Mass Exercise

Based on the previous evidence, one would not expect an effect of increased sympathetic nervous activity on blood flow to a small exercising muscle mass such as the human forearm, the flow requirements of which would not threaten the pumping capacity of the heart. However when such sympathetic elevation has been induced, either via stimulation of sympathetic nerves to active skeletal muscle (Thompson and Mohrman, 1983; Remensnyder *et al.*, 1962; Kjellmer, 1965; Peterson *et al.*, 1988; Donald *et al.*, 1970; Klabunde, 1986), lower body negative pressure

(LBNP) (Shoemaker *et al.*, 1997; Strandell and Shepherd, 1967), direct stimulation of the carotid sinus nerves (Vatner *et al.*, 1970) or upright vs. supine posture (Joyner *et al.*, 1990), evidence for sympathetic restraint is conflicting. Some investigators have observed attenuated blood flow with elevations in sympathetic activity (Thompson and Mohrman, 1983; Shoemaker *et al.*, 1997; Strandell and Shepherd, 1967; Peterson *et al.*, 1988; Joyner *et al.*, 1992; Joyner *et al.*, 1990). Others have observed virtually no effect during exercise (Remensnyder *et al.*, 1962; Kjellmer, 1965; Hansen *et al.*, 1996; Donald *et al.*, 1970), suggesting that a "functional sympatholysis" occurs. Numerous physiological mechanisms for such a functional sympatholysis have been clearly documented. Inorganic phosphate, acetylcholine, adenosine, acidosis and potassium have all been demonstrated to inhibit sympathetic neuro-transmission (Eboute *et al.*, 1987; Rorie *et al.*, 1981; Verhaeghe *et al.*, 1977) (for review see Shepherd (1983) and Shepherd and Vanhoutte (1981)). In the studies where sympathetic restraint was observed, it depended upon any one or a combination of the following factors: the exercise intensity (Shoemaker *et al.*, 1997; Joyner *et al.*, 1990; Joyner *et al.*, 1992), the level of sympathetic activity (Thompson and Mohrman, 1983; Strandell and Shepherd, 1967; Kjellmer, 1965), and at what point during the exercise blood flow was measured (Peterson *et al.*, 1988; Kjellmer, 1965; Joyner *et al.*, 1990). It appears that we still do not know what determines whether a sympathetic restraint or a functional sympatholysis dominates the local exercising muscle blood flow response.

Aim of Studies

The primary aim of the studies presented in this thesis was to provide insight into the regulation of blood flow to muscles at rest and at the onset of dynamic exercise. The model used was that of forearm exercise and therefore investigated primarily the adaptation of local blood flow control factors. Specifically, the issues of whether local venous pressure contributes to the downstream pressure determining muscle blood flow and whether elevated sympathetic nervous activity can blunt the vasodilatory control of muscle vascular conductance at rest and in exercise were examined.

Specific research questions in each of the studies in this thesis were as follows:

1. At the onset of exercise there is an immediate, rapid increase in blood flow followed by a second, slower adaptation phase. It has been suggested that the mechanical emptying of veins due to muscle contractions (the muscle pump) could account for the entire, initial rapid increase in muscle blood flow at the onset of exercise (Sheriff *et al.*, 1993). This hypothesis was tested in Paper I (Chapter II).

2. With changes in limb position relative to heart level, the local arterial-venous pressure gradient is altered by a hydrostatic effect and the emptying and refilling of the venous volume. The effect on both resting and exercising blood flow would depend on the relative contribution of arterial and venous pressure to the true upstream-downstream pressure gradient and on the response of the vasculature to both the local hydrostatic pressure effects and the influence of the veno-arteriolar reflex. The question of how changes in limb position relative to heart level affect limb blood flow was investigated in Paper II (Chapter III).

3. Increases in sympathetic nervous activity can result in constriction of blood vessels and

thereby contribute to the regulation of blood pressure during exercise. Such a vasoconstrictor effect in exercising muscle is in opposition with the local goal of vasodilation during exercise to meet the metabolic demands of the contracting muscle. Whether elevations in sympathetic nervous activity as part of the systemic pressure regulating response blunt the blood flow response in small muscle mass exercise where blood pressure is not threatened by the vasodilation of the exercising vascular bed, or whether there are local vasodilatory factors that can overcome the sympathetic influence and maintain the desired blood flow response was tested in Paper III and Paper IV (Chapter IV and V).

4. Immediately following exercise there is an initial hyperemia above that during exercise, which gradually returns to resting levels. This study addressed the question of whether this post exercise hyperemia was greater in magnitude than that during exercise due to a withdrawal of sympathetic vasoconstrictor activity present during exercise (Appendix I).

Methodology

Non-invasive techniques which provide beat by beat cardiovascular measures can play an important role in the assessment of muscle blood flow control mechanisms at the onset of voluntary exercise or alterations in limb position relative to heart level in humans. In the studies in this thesis, instantaneous blood flow was obtained by pulsed and echo Doppler ultrasound, while continuous estimates of arterial pressure were obtained via a photo plethysmographic finger cuff (Imholz *et al.*, 1990). Both methods have been used extensively in our laboratory over the past few years (Shoemaker *et al.*, 1996; Shoemaker *et al.*, 1997; Shoemaker *et al.*, 1996; Hughson *et al.*, 1997; Tschakovsky *et al.*, 1995; Shoemaker *et al.*, 1994). Figure 1.2 is an example of the output from present day Doppler technology which allows us to non-invasively observe the instantaneous blood flow in a feed artery to exercising muscle. The pulsatile nature of arterial flow is evident and flow can be quantified on a beat by beat basis, while characteristics of the blood pulse can shed light on the state of the vasculature that the feed artery supplies.

During alterations in limb position, forearm volume changes were assessed with a mercury in silastic rubber strain gauge to provide estimates of venous volume changes, while preliminary estimates of venous pressure were obtained by direct transducer measurement of venous pressure via catheterization of a vein draining the forearm at the level of the elbow. This section will describe the principles behind these techniques and their validation.

Blood Flow

The technique of Doppler ultrasound in the measurement of blood flow is based on the Doppler principle which states that when an observer is moving relative to a wave emitting source, the measured frequency differs from the emitted frequency. This difference is

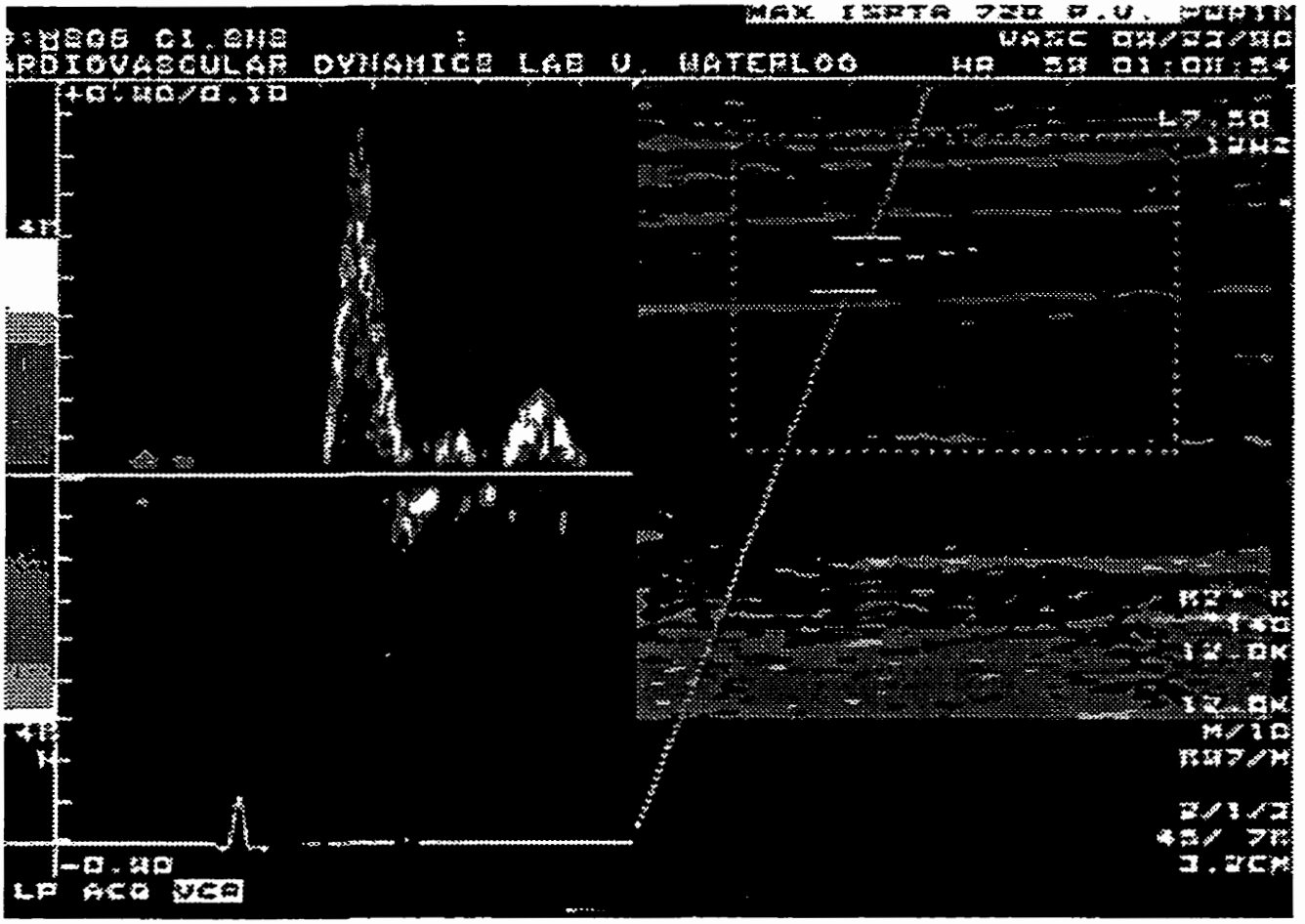

Figure 1.2 *An example of the resting forearm blood flow response through the brachial artery (top left) and an image of the brachial artery (top right) obtained with pulsed and echo Doppler. The scale to the left indicates velocity. The lack of arterial inflow at rest during diastole is apparent.*

proportional to the relative velocity of the wave source and the observer. In measuring blood flow, the Doppler principle is employed by directing ultrasonic energy (MHz level) to intersect the blood vessel of interest. Vibration of a piezoelectric crystal provides the ultrasonic energy. This crystal will vibrate when connected to a source of electrical energy and, conversely, will create electrical energy when it is subjected to mechanical vibrations as are caused by the reflected sound waves (Fronek, 1989). Red blood cells will reflect these sound waves, effectively acting as a "moving wave source", and the frequency of the sound waves will be shifted from that emitted by the crystal. The type of ultrasound probe used in our laboratory is that of pulsed Doppler. This means that the same crystal acts as both a receiver and transmitter of the ultrasound. Short pulses of ultrasound are followed by short pauses during which the returning ultrasound is received by the crystal. This system can effectively "focus" its attention on ultrasound returning to the crystal from a specific depth and sample area, where depth of focus is determined by the timing of the receiving period and the sampling volume or "gate" is determined by the duration of that sampling period (Fronek, 1989).

Conversion of the reflected ultrasonic energy to a blood velocity is based on the proportionality of the frequency shift of the reflected ultrasound from the emitted ultrasound with velocity. This is described by the equation

$$v = f_D \cdot C/2f_t \cdot \cos(\theta) \qquad \text{Eqn. 2}$$

v = velocity

f_D = Doppler shift frequency (Hz)

C = velocity of sound in tissue and blood in cm/s

f_t = probe transmission frequency

θ = angle of insonation of the ultrasound beam

The angle θ used to calculate blood velocity in the experiments in this thesis is 45° based on the probe assembly and previous identification that the brachial artery at the sight of measurement at the elbow is parallel to the skin surface (Shoemaker *et al.*, 1996). The returning ultrasound consists of a spectrum of frequencies representing the differing velocities of red blood cells (Figure 1.2). Velocity direction is detected by mixing the received signal with two reference signals shifted in phase relative to each other by 90°. Two signals are therefore formed which are shifted by 90° positive or negative corresponding to the direction of blood flow relative to the emitted ultrasonic energy. Resting forearm vascular resistance and muscle contraction compression of the forearm vasculature during exercise both result in retrograde flow during part of the cardiac cycle, therefore this feature of detecting directional flow is particularly important.

The application of Doppler for imaging operates on the same principle of reflected sound. However it takes advantage of the fact that different tissue structures absorb and reflect ultrasound to different degrees . An array of detecting crystals can therefore effectively "map" tissue structures depending on the intensity of the reflected ultrasound, thereby providing a picture of the brachial artery walls (Figure 1.2). This is done in B-mode or "brightness mode" imaging whereby the intensity of the reflected signal is represented by the brightness of a dot on a video screen. This information can be recorded on videotape for later measurement of arterial diameter.

For this thesis, blood flow was measured as follows. A 4.0 MHz flat probe of a pulsed Doppler unit (model 500V, Multigon Industries, Mt. Vernon NY) was positioned over the brachial artery at the level of the antecubital fossa and taped to the skin (Shoemaker *et al.*, 1996;

Tschakovsky *et al.*, 1995). During exercise, the probe could be manipulated by the operator to maintain the optimum signal as determined by constant auditory and visual feedback. Mean blood velocity (MBV) was obtained from the spectra of frequency signals processed by a mean velocity analyzer which provided a weighted mean of the velocities based on the intensity of the signal corresponding to each frequency shift in the Doppler spectrum (Micco, 1989). The MBV was collected at 100 Hz on a dedicated computer system. For the purpose of beat by beat analysis of cardiovascular variables both heart rate and arterial blood pressure were also collected on separate channels. Calibration of the Doppler signal was performed prior to each experiment. The mean velocity analyzer provided a frequency signal representing the frequency shift obtained for the 4 MHz probe at 0° insonation angle and a blood velocity of 1 m/s and -1 m/s.

At least 2 trials were performed in each experimental condition. Each beat was defined to begin in time with the R-wave of the ECG and end at the next R-wave (R-R interval) and converted to an average velocity by integrating the area under the beat velocity curve. For rhythmic exercise conditions, the beat by beat data were averaged across trials over the time required for a complete contraction/relaxation cycle. In experiments where no contractions were being performed, the data were linearly interpolated to provide a data point at every second and then averaged across trials. Analysis of relaxation phase blood flow during rhythmic contractions involved the manual placement of beat markers on beats unaffected by contraction, which were then averaged across corresponding relaxation phases for all the trials of a given condition.

Brachial artery diameters were obtained by continuous measurement of the brachial artery a few centimeters proximal to the site of velocity measures during one of the trials in each experimental condition using a linear echo Doppler 7.5 MHz hand held probe (model SSH140A,

Toshiba Inc., Tochigi-Ken, Japan). The data were stored on videotape for subsequent frozen screen analysis of brachial artery diameter. Previously in our laboratory we have demonstrated that brachial artery diameter does not differ between the sites selected for image and velocity measurement (Shoemaker *et al.*, 1996). For experiments involving rhythmic exercise, a series of measurements were made from frozen screen images (each diameter value was the average of three separate measurement caliper placements on the frozen screen). These calipers could be adjusted in 0.1 mm increments. Continuous brachial artery diameter estimates were then obtained by a line of best fit of the diameter data. Finally, forearm blood flow could then be calculated as $FBF = MBV \cdot \pi r^2$. Shoemaker et al. (1996) have shown the day to day reproducibility of the these measurements to have a coefficient of variation of 2-4%.

Blood Pressure

A finger plethysmograph (Finapres 2300, Ohmeda, Englewood, CO) was used to measure arterial blood pressure on a beat by beat basis. Briefly, this system has an infrared emitting diode in the finger cuff and a detector immediately opposite, such that when the cuff encircles the finger, light from the diode travels through the finger and the amount reaching the other side can be detected. Absorption of the light is proportional to the distance through the finger that it must travel. With each heart beat, the change in vessel transmural pressure causes the finger to "swell" proportionally. Matching the changes in transmural pressure via instantaneous, equal increases in cuff pressure therefore maintains finger volume and provides continuous estimates of arterial pressure. Comparisons with direct arterial blood pressure measures indicate a good agreement (Imholz *et al.*, 1990). Along with MBV and heart rate, blood pressure was collected at 100 Hz on a dedicated computer. Integration of the area under the curve provided mean arterial pressure.

Venous Pressure

Venous pressure was measured in an antecubital vein draining the muscles via a catheter (20 gauge Angiocath) inserted in a retrograde fashion with a tygon line connected to a pressure transducer (Gould P23 Db series, Gould Inc., Oxnard Ca.) positioned at the height of the catheter tip. A 2-point calibration was obtained by measuring atmospheric "0" and then providing a hydrostatic column of water to 50 cm. Appropriate conversions of cm H_2O to mmHg (1 cm H_2O=0.7355 mmHg) were made for expression of the data. The sampling frequency for collection on a dedicated computer was 100 Hz, however for analysis the value at each R-wave marker was used.

Venous Blood Gases

This technique was used in Chapter IV. 1 ml blood samples were obtained from an antecubital vein draining muscles of the forearm. Samples were obtained anaerobically in heparinized syringes and immediately mixed and placed in an ice bath. Analysis (Nova StatProfile 9 Plus, Nova Biomedical Canada, Mississauga, ON) for venous blood gases began within minutes of the first blood sample being collected. Calibration of the analyzer was performed at regular intervals during the experiment. The analysis system provided the calculated % saturation of O_2 using Hb-O_2 saturation curves based on pH and CO_2 measures in the venous blood. Total venous O_2 (C_vO_2) content was calculated by the analyzer system from the equation

C_vO_2 (ml/100ml) = 1.39 ml O_2/gHb · [Hb] (g/100ml) · (%O_2 saturation/100) + 0.003 PO_2 Eqn. 3

[Hb] = blood hemoglobin concentration

PO_2 = partial pressure of dissolved oxygen

To obtain arterial - venous oxygen difference (a-vDO_2), the arterial oxygen content was

estimated with the same equation, with the venous [Hb] used to represent arterial [Hb] under the following assumptions:

1. Arterial $\%O_2$ saturation was 97%, as has often been measured in our laboratory under resting conditions, and did not change during the experiment. The exercise performed was only moderate single forearm hand grip exercise that provided little challenge to the central cardiovascular system. Therefore, it is safe to assume that arterial saturation should not change.
2. Venous [Hb] is equivalent to arterial [Hb].

Forearm $\dot{V}O_2$ was then calculated as the product of forearm blood flow and a-vDO_2.

One concern with this method of determination of forearm $\dot{V}O_2$ would be the integrity of the blood samples that were analyzed some time after collection. There have been indications that, despite immediately placing samples on ice, the PO_2 in blood samples from subjects breathing hyperoxic gas can fall rapidly over time (Knight *et al.*, 1993). However, others have not found a significant decay in samples taken from subjects breathing hypoxic and normoxic gas (Roca *et al.*, 1989). Values obtained in this study were consistent with the expected metabolic response, as indicated by the similarity of calculated forearm $\dot{V}O_2$ between control and LBNP conditions. As well, numerous samples were analyzed for a second time after a delay of ~1 hour and no difference in PO_2 was observed, agreeing with previous findings (Roca *et al.*, 1989).

Another concern is the distribution of outflow at the site of the venous sampling in relation to the total inflow measured at the brachial artery. Figure 1.3 illustrates the general venous drainage pattern observed from examination of 1 cadaver and several echo Doppler imaging sessions which confirmed the deep vein branch merging at the site of catheterization. Generally,

deep branches draining forearm flexors and extensors (both muscle groups would be used in handgrip contractions) and superficial skin veins meet at a junction shown in Figure 1.3. Catheter orientation anterograde with venous flow means that the catheter tip would have been "downstream" of this junction, while retrograde placement (used in the study in Chapter IV) means that the catheter tip was potentially "upstream" of this junction in a deep vein. Even deep veins receive blood from skin veins of the hand (Corcondilas *et al.*, 1964), however prior testing in 1 subject in our laboratory has shown that positioning of the catheter anterograde vs. retrograde in the same vein used in this study results in dramatically different measures of venous PO_2 with alterations in skin blood flow at rest. With the forearm cool vs. heated the forearm blood flow was 29.7 vs. 57.8 ml/min. Venous measures of PO_2, $\%O_2$ saturation and the subsequent calculated forearm $\dot{V}O_2$ for retrograde cool vs. heated forearm were 32 vs. 38.5 mmHg, 58 vs. 70.6 $\%O_2$ sat., and 2.3 vs. 1.6 ml/min respectively. The same measures for anterograde catheter placement were 40.6 vs. 74.4 mmHg, 70 vs. 93.7 $\%O_2$ sat., and 3.1 vs. 0.4 ml/min. It is clear from the increase in venous $\%O_2$ saturation that both catheter placements receive skin blood flow contributions, however the retrograde catheter placement clearly reduces the skin blood flow contribution, and additionally, the maintenance of a cool arm would appear to minimize skin flow contribution at this site.

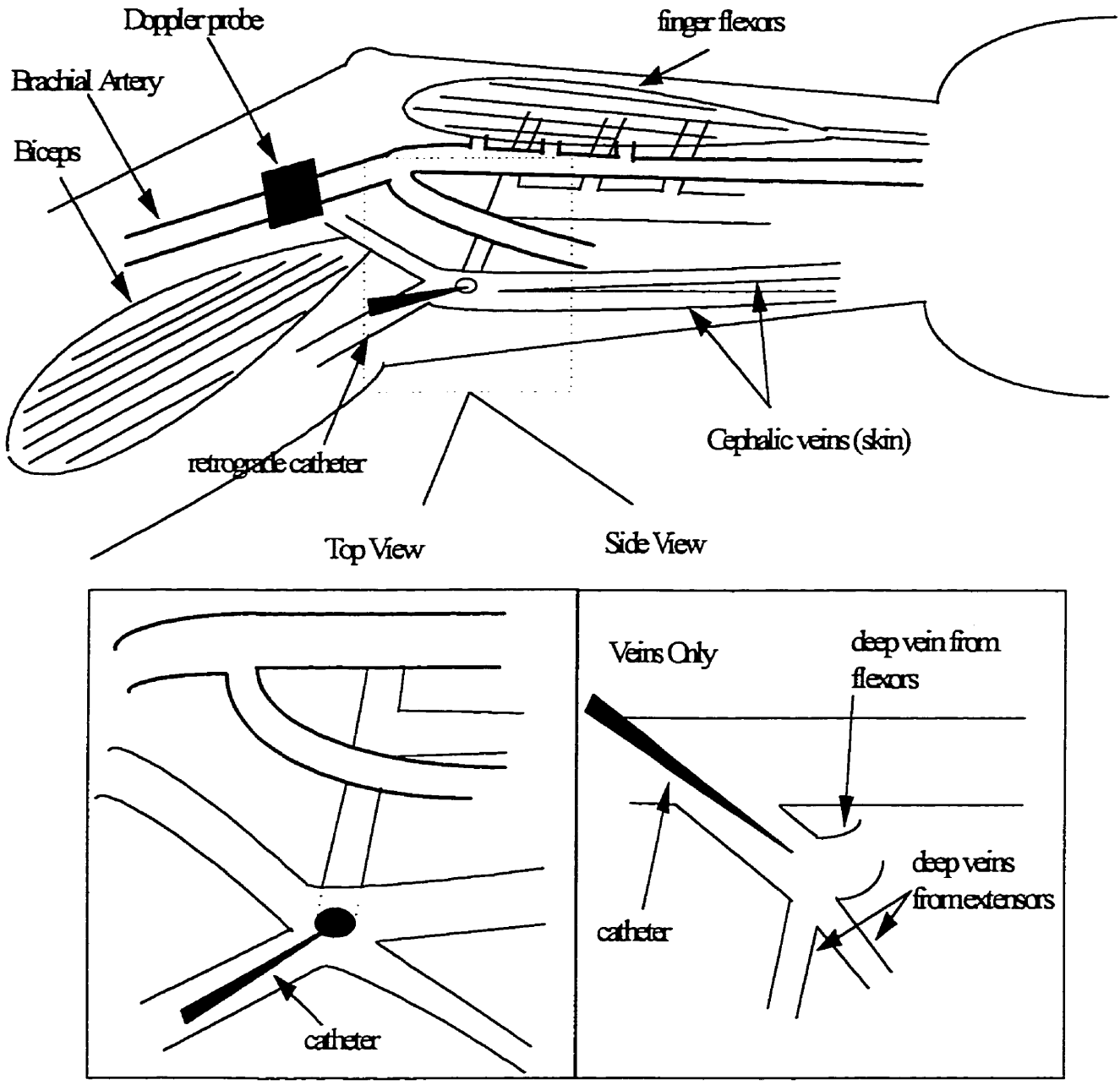

Figure 1.3 *This is a schematic illustration of arterial and venous blood vessels obtained from the examination of a cadaver and echo Doppler imaging in a number of subjects . The main point to notice is the confluence of venous drainage at the site of catheterization. The side view depicts optimum catheter placement, but this could not be confirmed. Nevertheless, it was likely the case given the venous oxygen content values obtained at rest.*

Strain Gauge Plethysmography: Forearm Volume and Blood Flow

Strain gauge plethysmography was used to determine changes in arm volume as an estimate of changes in vascular volume. Changes in arteriolar and capillary volume with altered pressure will be small because the arteriolar circulation is relatively non-compliant while changes in venous volume will be much larger due to its high compliance (Tyberg and Baker, 1993). Alterations in arm volume that are due to fluid filtration are delayed and appear to be quite small (Ardill *et al.*, 1968). Therefore, acute changes in limb volume on movement to and from the dependent position reflect predominantly changes in venous volume. This application for strain gauge plethysmography has been used previously to investigate the behaviour of the capacity vessels (Bevegard and Shepherd, 1966; Barendsen and van den Berg, 1984; Ardill *et al.*, 1968). Briefly, a mercury in silastic rubber strain gauge was positioned around the arm at the area of largest circumference. Electrical resistance of the strain gauge changes in proportion to its length such that changes in arm circumference at the site of measurement result in detectable changes in voltage across the electrical circuit of the strain gauge plethysmograph. The principle of measuring arm volume based on circumference changes is based on the proportional relationship between changes in circumference at any point along the length of any cylinder and changes in volume. This is however based on the assumption that changes in volume are radial in direction and proportional along the length of the entire cylinder (Whitney, 1953). Assuming this proportionality, changes in arm volume are then expressed as a % (ml/100ml). Forearm blood flow is obtained by simply inflating a venous occlusion cuff (50 mmHg was the inflation pressure used in the study in Chapter IV) positioned proximal to the elbow such that venous outflow is occluded but arterial inflow is presumably unaffected. The increase in forearm volume over time

then represents forearm blood flow. It should be mentioned here that there is evidence that cuff inflation may reduce arterial inflow by reducing arterial diameter (Hiatt *et al.*, 1989) and increasing venous pressure (Tschakovsky *et al.*, 1995). Calibration of this system is obtained by an internally generated voltage proportional to a 1% change in strain gauge length to provide a 2 point calibration (0 and 1 ml/100ml). Data were collected at 100 Hz on a dedicated computer and the value at each R-wave was used for data analysis.

CHAPTER II

Vasodilation and muscle pump contribution to immediate exercise hyperemia

(Published: *Am J Physiol* (1996). **271**:H1697-H1701,with added appendix)

ABSTRACT

A rapid (within 0-5 s) increase in skeletal muscle blood flow has been demonstrated following muscle contraction, yet the mechanism remains unresolved. Recently, it was suggested that the entire rapid exercise hyperemia could be attributed to the mechanical muscle pump effect. Other evidence indicates that the muscle pump cannot increase arterial flow. We measured human forearm blood flow with the arm positioned above or below heart level during *1*) simulation of rhythmic muscle pump function via repeated inflation/deflation of a forearm cuff to 100 mmHg to achieve mechanical emptying of forearm veins, and *2*) 1-s single cuff inflations, 1-s voluntary forearm contractions and 1-s contractions performed within a cuff inflation. Rhythmic cuff inflation increased blood flow with the arm below heart level ($P<0.05$) but not above. Flow following single contractions was higher than flow following cuff inflation within 2 s ($P<0.05$). Peak flow increases due to a single mechanical venous emptying (7.7 ± 0.7 ml/100ml/min) could account for 60% of the peak flow increase due to muscle contraction (12.8 ± 1.0 ml/100ml/min) with the arm below heart level, while above heart level mechanical venous emptying accounted for 46% of the flow increase due to contraction (3.0 ± 0.4 ml/100ml/min vs. 6.5 ± 0.6 ml/100ml/min). We conclude that a functional muscle pump does exist in the human forearm in vivo, but that a rapid vasodilation detectable by 2 s also contributes to the early exercise hyperemia.

Key words: blood flow, circulation, vascular control, forearm exercise

INTRODUCTION

Despite direct (Marshall and Tandon, 1984) and indirect (Lind and Williams, 1979; Leyk *et al.*, 1994; Corcondilas *et al.*, 1964) indications that vasodilation might play an immediate role in the hyperemia at the onset of exercise, it has recently been stated that the muscle pump is sufficient to account for the increase in blood flow during the first 5 s of exercise (Sheriff *et al.*, 1993). The theoretical basis for the muscle pump's effect on flow through any vascular bed is described by a form of Ohm's law for the circulation

$$\dot{Q}_a = \Delta P \cdot VC$$

where arterial inflow ($\dot{Q}_a$) is determined by the upstream (arterial) to downstream (venous) pressure difference (ΔP) across a vascular bed and the vascular conductance (VC) of that bed. Pollack and Wood (1949) found that muscle contraction effectively increased ΔP by squeezing blood out of the venous capacitance vessels, thereby lowering venous pressure upon muscle relaxation. Folkow et al. (1970) proposed that this gain in pressure gradient could elevate flow. In addition, it has been proposed that pressure in the venules might become negative due to the pulling open of tethered veins by relaxing muscle (Laughlin, 1987), further contributing to a widening of ΔP. Although support for the function of the muscle pump is extensive as reported by Laughlin (1987), other researchers have interpreted evidence from *in situ* dog hindlimb muscle preparations to mean that the micro circulation behaves like a vascular waterfall and that the muscle pump therefore could not assist blood flow by its effect on local venous pressure (Permutt and Riley, 1963; Naamani *et al.*, 1995; Jackman and Green, 1990; Braakman *et al.*, 1990).

Given this considerable controversy, we conducted two experiments with 10 healthy human

subjects to determine which mechanisms are responsible for the immediate increase in blood flow to contracting muscle. In the first experiment, the mechanical effect of rhythmic muscle contractions was simulated via rhythmic inflation/deflation sequences (pumping) for 1 min using a forearm cuff. In the second experiment, single muscle contractions were compared with single-cuff inflations and with single contractions completed while the cuff was inflated to quantify the relative contributions of mechanical and possible vasodilatory mechanisms in the immediate (0-5 s) hyperemic response.

METHODS

Subjects

Ten healthy, young male subjects (25.8 ± 1.1 yr, mean ±SE) volunteered for this study, and gave written consent on a form approved by the Office of Human Research of the University once they had received full written and verbal details of the experimental protocol and any potential risks involved.

Experimental Design

Repeated cuff inflations. Ten normal subjects lay supine, with the arm supported in an extended position at an angle from the horizontal of either 50° above (Above) or 50° below (Below) heart level to induce differences in muscle perfusion pressure (mean difference at mid forearm level approximately 30 mmHg). The order of Above or Below experiments was counterbalanced between subjects. To simulate the mechanical compressive effect of rhythmic muscle contraction, a cuff wrapped around the right forearm was rapidly inflated to 100 mmHg with an inflation/deflation rhythm of 1-s/2-s. Three 3-min trials per subject were performed in each arm position, consisting of 1 min of rest prior to and following 1 min of rhythmic cuff inflations.

Single contraction or cuff inflation. Subject position was as described above. The blood flow response to a single 1-s cuff inflation was compared with a single voluntary handgrip contraction. For contractions, an 8.6 kg weight was raised and lowered 5 cm in time with a 1-s signal light. To determine if cuff inflation and muscle contraction had similar mechanical effects and whether release of a muscle contraction *per se* might induce a negative venous pressure compared with simply releasing mechanical muscle compression, a single muscle contraction was

also performed for 1 s with the cuff being inflated immediately before and deflated immediately after this contraction.

Data Acquisition

Heart rate, mean arterial perfusion pressure (MAP) and brachial artery mean blood velocity (MBV) were measured beat-by-beat. MAP was measured at mid-forearm level using a photoplethysmograph finger blood pressure cuff (Finapres, Ohmeda 2300, Englewood, CO) (Imholz *et al.*, 1990) on the contra-lateral hand which was positioned with the finger cuff was at mid-forearm level of the cuffed arm. Forearm blood flow was obtained beat by beat as the product of MBV and arterial cross-sectional area. Blood velocity was measured with a 4-MHz pulsed Doppler ultrasound probe (Multigon Industries, Model 500V, Mt. Vernon, NY) fixed to the skin over the brachial artery in the antecubital fossa region of the right elbow (Tschakovsky *et al.*, 1995). Probe angle relative to the skin was 45°. Arterial cross-sectional area was measured by echo Doppler (Toshiba Model SSH-140A, Tochigi-Ken, Japan) at rest prior to the cuff inflations or contractions using a linear 7.5 MHz probe operating in B-mode. Imaged data were stored on video tape (Panasonic Model AG-7300) for subsequent analysis. Mean arterial diameter was determined as the average obtained from a total of ten frozen screen arterial diameter images of the brachial artery during diastole. All diameter measurements were made by the same operator. Forearm volume was obtained by water displacement. All data were saved continuously at 100 Hz via analog-to-digital conversion (Metrabyte DAS-16, Taunton, MA) on a personal computer. For *repeated cuff inflations*, each of the three trials was averaged into 3-s bins corresponding to cuff inflation/deflation cycles and then averaged across trials to determine the mean subject response. For the single contraction or cuff inflations, five trials per subject

were performed in each experimental condition. All trials were time-aligned to the release of cuff inflation or muscle contraction. The MBV data were then interpolated between heart beats, providing data every second to allow averaging within and across all 10 subjects.

Statistical Analysis

For the first experiment, the effects of arm position and time (from rest through cuff inflations and recovery) were evaluated by two-way repeated measures analysis of variance (ANOVA). Further investigation into main effects was performed by repeated measures one-way ANOVA. For the second experiment, the effect of arm position and contraction protocol (cuff inflation, contraction, and contraction within cuff inflation) were investigated using repeated measures ANOVA. The level of significance for ANOVA was set at $P<0.05$, with significant differences being further analyzed with Student-Newman Keuls post hoc testing. All data are presented as means ± SE.

RESULTS

Repeated cuff inflations

When the forearm was below heart level, blood flow increased acutely by 60% in response to rhythmic cuff inflation/deflation and then fell slightly, but remained significantly elevated above rest (35%) ($P<0.05$) (Figure 2.1). When cuff inflation ceased, flow gradually returned to resting levels, likely determined by the refilling of the venous volume. With the arm supported above heart level, flow to the forearm was not affected by rhythmic cuff inflation. Mean arterial perfusion pressure at mid forearm level prior to cuff inflations (107 ● 4 mmHg below and 73 ● 3 mmHg above heart level) did not change with cuff inflations and therefore could not explain the flow response. Direct measures of venous pressure from a catheter at the level of the elbow in one subject showed resting levels to be approximately 32 mmHg with the arm below the heart and 0 mmHg with the arm above the heart. This confirmed that the magnitude of the gravitational effect seen on the arterial side of the circulation also occurred on the venous side, suggesting similar effects on resting venule pressure.

Single contraction or cuff inflation

Blood flow due to muscle contraction was elevated compared with cuff inflation as early as 2 s after the release of the contraction or cuff (Figures 2.2 and 2.3) ($P<0.05$). Peak blood flow responses occurred 4-5 s after muscle contraction, and were delayed compared with cuff only deflation (1-3 s, $P<0.05$, Figure 2.2). The peak change in flow above rest in the below heart condition was greater in cuff + contraction (15.8 ± 1.5 ml/100 ml/min) than contraction only (12.8 ± 1.0 ml/100 ml/min), which in turn was greater than cuff only (7.7 ● 0.7 ml/100 ml/min) ($P<0.05$). When the arm was above the heart, peak blood flow for cuff + contraction (7.8 ± 0.8

ml/100 ml/min) and contraction (6.5 ± 0.6 ml/100 ml/min) were significantly greater than cuff only (3.0 ± 0.4 ml/100 ml/min) ($P<0.05$).

Individual blood velocity profiles (see Figure 2.3) were consistent with a combined mechanical and vasodilatory effect of contraction. Flow increased immediately in all conditions, being greater following contraction compared to mechanical venous emptying in both the below and above tests, with the peak cuff effect occurring earlier than the peak contraction effect.

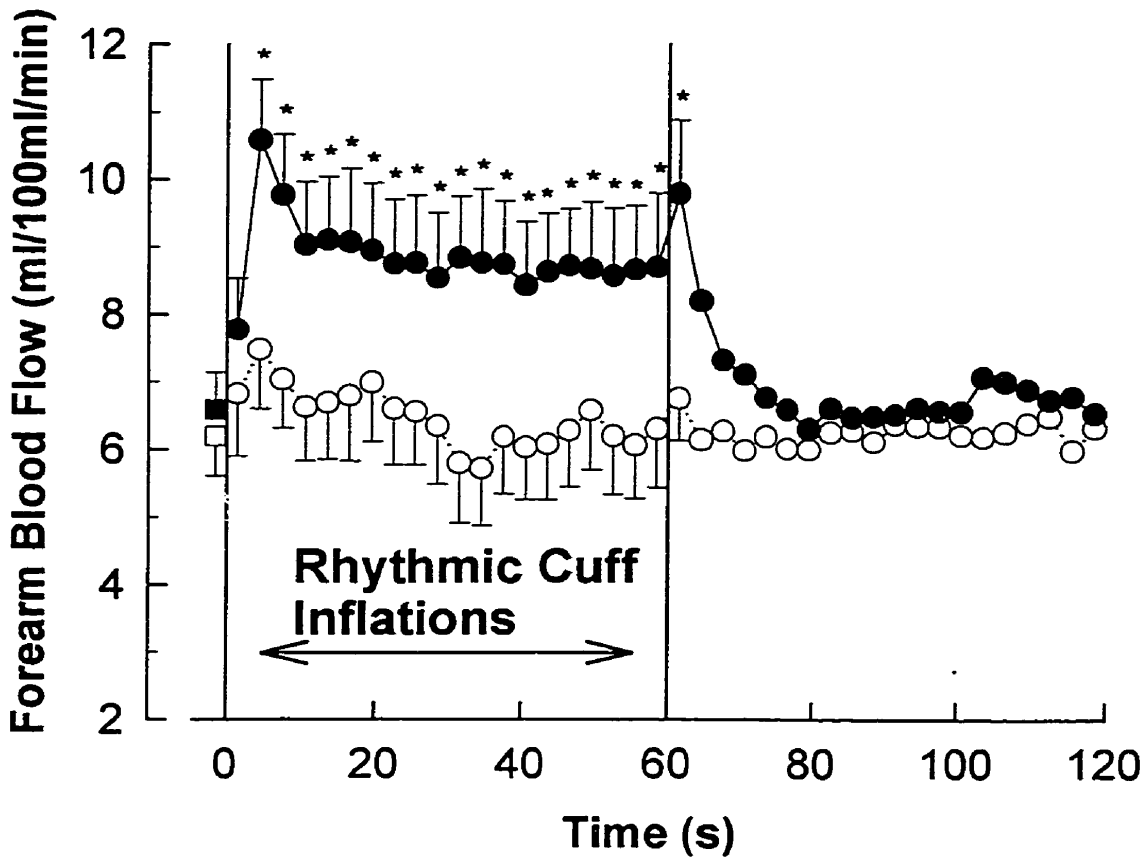

Figure 2.1 *Forearm blood flow responses to 1 min of rhythmic forearm cuff inflation to 100 mmHg (1-s inflation/2-s deflation) with the arm above (○) and below (●) heart level (mean ±SE of 3-s periods). * Significantly different (P<0.05) from 10 s resting average: □, above heart, ■, below heart.*

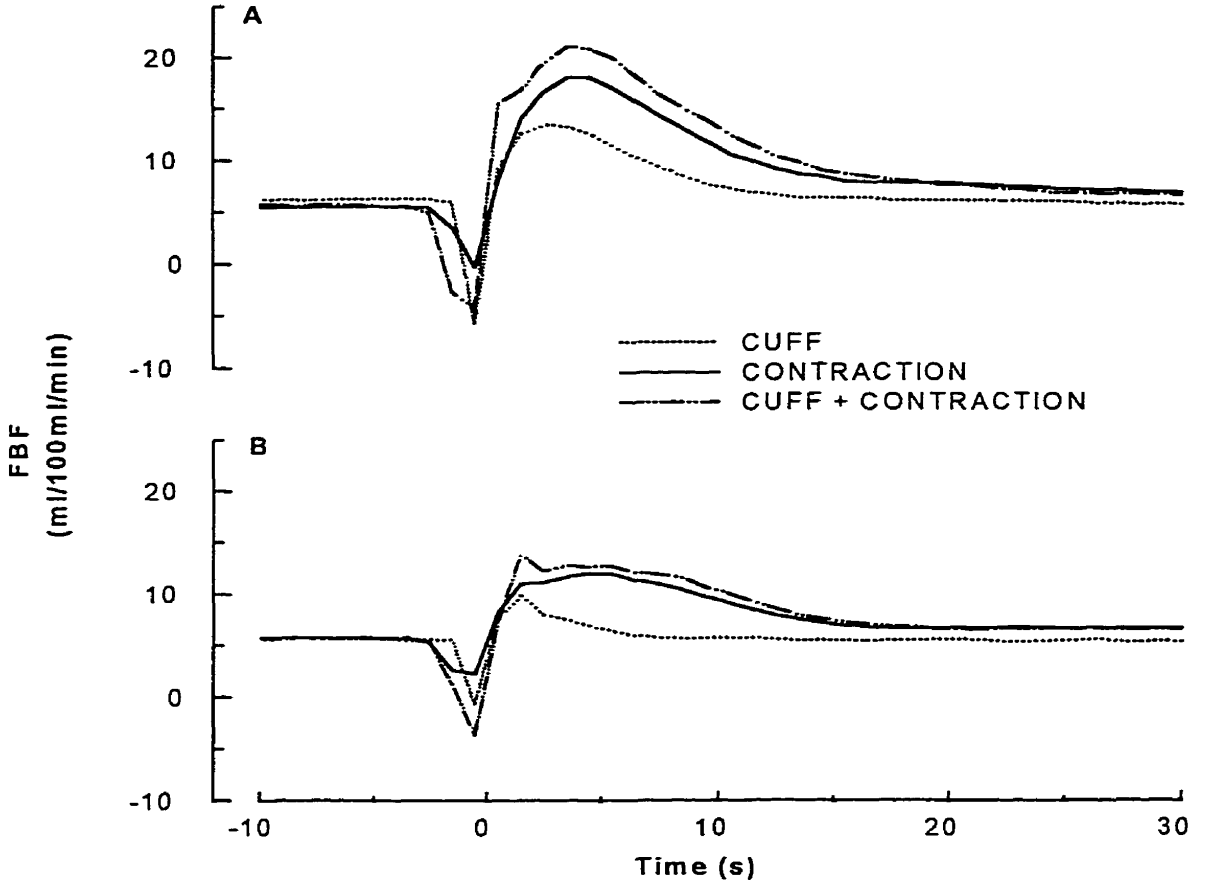

Figure 2.2 *Forearm blood flow (FBF) response, averaged across all 10 subjects, to a single 1-s cuff inflation (Cuff), a single contraction (Contraction), and a single contraction within a cuff inflation (Cuff + Contraction) with the arm below (A) and above (B) the heart. Compressive force of cuff inflation and muscle contraction induced retrograde arterial flow. On cuff deflation or muscle relaxation (time = 0), an initial surge of blood back into the arm, likely refilling the arterial volume, was followed by continued flow increases in all conditions. As early as 2 s following contraction, flow was higher compared to cuff inflation ($P<0.05$). Peak flow and duration of elevated flow were greater following contraction compared to cuff inflation in A and B ($P<0.05$).*

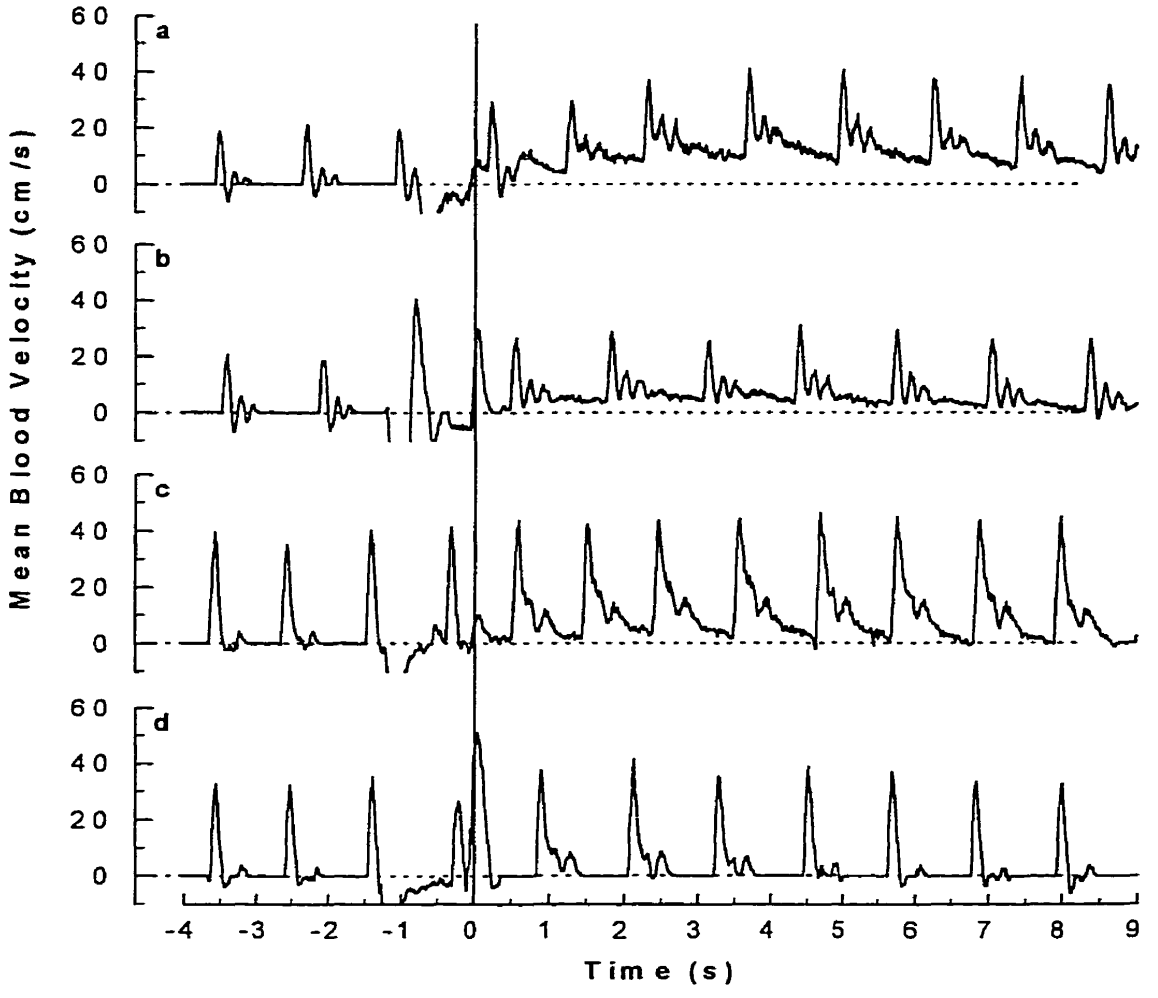

Figure 2.3 *Example from a single subject of beat-by-beat arterial inflow blood velocity waveforms in response to forearm contraction, arm below heart level (A); cuff inflation, arm below heart level (B); forearm contraction, arm above heart level (C); cuff inflation, arm above heart level (D). Release occurred at time=0 s. In contrast to resting condition during which flow occurred only during systole, post contraction conditions showed flow during systole and diastole, with greater flow in the below heart tests. Compared with post contraction responses, increased blood velocity after cuff inflation was of a smaller magnitude and duration.*

DISCUSSION

This study was prompted by the recent controversy about the mechanism responsible for the immediate increase in blood flow with muscle contraction. Although evidence has been presented that a very rapid vasodilation upon muscle contraction occurs both *in situ* (Marshall and Tandon, 1984) and *in vivo* (Lind and Williams, 1979; Leyk *et al.*, 1994; Corcondilas *et al.*, 1964), some have argued that such rapid vasodilation is unlikely and that the muscle pump mechanism alone accounts for the initial (0-5 s) flow increase (Sheriff *et al.*, 1993). Furthermore, other researchers have interpreted evidence from *in situ* dog muscle preparations to suggest that the micro circulation is best modeled as a vascular waterfall, and therefore muscle pump effects on local venous pressure cannot affect arterial inflow (Permutt and Riley, 1963; Naamani *et al.*, 1995; Jackman and Green, 1990; Braakman *et al.*, 1990). Evidence from this study strongly suggests that both a functional muscle pump and rapid vasodilation act in concert to initiate the increase in blood flow during the first 5 s of exercise in human skeletal muscle.

Repeated cuff inflations

At rest we observed flows that were not different between arm-above and arm-below heart positions, which is consistent with earlier studies in cat muscle (Folkow, 1952; Folkow, 1949). During rhythmic cuff inflations, the initial 60% increase in blood flow with the arm below heart level was followed by a slight decrease to levels 35% above rest. This delayed decrease could be explained by an autoregulatory response of the forearm vasculature as seen elsewhere in experimental models where flow is artificially elevated while metabolic rate is maintained (Shepherd, 1983). After the cessation of rhythmic cuff inflations with the arm below the heart, flow momentarily increased and then decreased towards resting levels, likely reflecting the

refilling of the venous volume. It is important here to remember that blood flow was reported as the average for a complete cuff inflation and deflation cycle (3 s). Therefore, the first data point following the end of cuff inflations represented a 3-s flow average in which there was no muscle compression to impede blood flow, resulting in the observed greater flow over that time in the arm below heart condition compared to flow during the previous rhythmic cuff inflations.

It might be argued that a myogenic vasodilation in response to reduced transmural pressure elicited by cuff inflation could explain the observed increases in blood flow with the arm below heart level. Muscle compression has in fact been used previously to investigate the role of such a myogenic response in exercise hyperemia. Mohrman and Sparks (1974) used a similar cuff model to ours on an isolated dog gastrocnemius preparation, and they attributed their cuff-induced flow increases following inflation to myogenic vasodilation in response to lowered transmural pressure. However others have failed to find a similar effect (Lind and Williams, 1979; Bacchus *et al.*, 1981) and have discounted a myogenic contribution. That a myogenic vasodilation might explain the flow increase with the arm below the heart seems unlikely in this study for two reasons. First, if muscle compression per se elicited a vasodilation, we should have observed an elevation in flow with the arm above heart level during the rhythmic cuff inflations, albeit to a lesser degree. In fact, no net increase in flow was observed. Secondly, if one looks at the flow response upon cessation of rhythmic contractions, a transient increase followed by a gradual decrease in blood flow to resting levels when the arm was below the heart can be observed, whereas no changes in flow are evident when the arm is above the heart. Such elevations in forearm blood flow with the arm below heart level are consistent with a mechanical effect of muscle contraction that is dependent on initial venous pressure (Folkow *et al.*, 1970), where the degree of contraction-induced

emptying of the veins results in proportional increases in ΔP, under normal in vivo conditions. This conflicts with conclusions drawn from pump-perfused, isolated dog hindlimb preparations in which changes in venous pressure did not affect arterial inflow (Naamani *et al.*, 1995; Jackman and Green, 1990; Braakman *et al.*, 1990) and suggests that caution be used in interpreting such models with respect to the in vivo condition.

Single contraction or cuff inflation

In this experiment, we investigated the possible contribution of vasodilation to the immediate hyperemia following a brief muscle contraction. The peak flow effect following a single contraction was always greater than that induced by cuff inflation within the same arm position (Figures 2.2 and 2.3), suggesting that a rapid vasodilation was acting in concert with the muscle pump. In fact, blood flow was significantly elevated following contraction compared to cuff inflation within 2 s following relaxation or cuff deflation (Figures 2.2 and 2.3). It is possible that the contraction of skeletal muscle was more effective at pumping blood from the veins within the muscle than the cuff inflation. However, the even higher flow achieved following the single contraction within a cuff inflation in the arm-below condition (Figure 2.2) indicated that the cuff likely added to the mechanical effect of muscle contraction, possibly due to the additional effect of the cuff on the veins of the skin. It has previously been suggested that negative venous pressure, uniquely due to active opening of veins during muscle relaxation, might contribute to the mechanical muscle pump effect (Sheriff *et al.*, 1993; Laughlin, 1987). The muscle contraction performed while the cuff was inflated allowed direct testing of this hypothesis. Active opening of veins was expected to result in a greater flow following contraction only compared with contraction during a cuff inflation. This was not found. Thus it seems unlikely, for this exercise

model at least, that such a mechanism has contributed to the flow increase.

For a given arm position, above or below, mean arterial perfusion pressure was unchanged with single contractions or cuff inflations. Therefore, the changes in blood flow could be taken to represent changes in virtual vascular conductance as defined by Rowell (1993) and Sheriff and colleagues (1993). True forearm arterial vascular conductance ($VC = \dot{Q}_x/\Delta P$) requires knowledge of the pressure gradient across the vascular bed in question. Because it is impossible to measure venular pressures, the virtual vascular conductance value is obtained by dividing blood flow by mean perfusion pressure at heart level. Changes in virtual conductance can then be due both to changes in resistance vessel diameter and changes in the local pressure gradient across the muscle vascular bed resulting from venous emptying due to muscle contraction. The current experiments allow us to argue that both mechanical and vasodilatory effects contribute to the increases that could be calculated for virtual vascular conductance.

With the arm below heart level, the immediate flow increase was greater than with the arm above, regardless of whether the muscle pump was acting alone or in concert with vasodilation, underlining the effect of local perfusion pressure on the ability of flow to adjust to muscle metabolic demand. Observations of faster time to peak flow following cuff inflation compared to either contraction or cuff + contraction were also consistent with an early mechanical effect following cuff inflation and with the cuff effect altering venous pressure, while muscle contraction also induced vasodilation.

In summary, this investigation revealed a contribution to the immediate flow increase following muscle contraction via a muscle pump effect, but this did not account for the total increase in flow with muscle contraction. Rather, as has been suggested by in vivo (Lind and

Williams, 1979; Leyk *et al.*, 1994; Corcondilas *et al.*, 1964) and in situ (Marshall and Tandon, 1984) studies, rapid vasodilation must occur at exercise onset. Further research is needed to identify which mechanism(s) can provide an immediate link between muscle activation and increases in blood flow.

Appendix

This appendix contains a response to some of the criticisms of this study that have been raised at various scientific conferences. It includes further analysis of the data (Figure 2.4) supporting the original interpretation of a combined muscle pump and vasodilation following a single voluntary contraction.

We compared the beat-by-beat forearm blood flow response to a single, 1 s dynamic forearm contraction with the response to a single, 1 s forearm cuff inflation (cuff pressure = 100 mmHg). Cuff inflation was used to simulate the mechanical venous emptying effect of contraction. Contraction resulted in a greater elevation in blood flow than cuff inflation and led us to conclude that there was "a contribution to the immediate flow increase following muscle contraction via a muscle pump effect, but that this did not account for the total increase in flow. Rather, as suggested by in vivo (Lind and Williams, 1979; Leyk *et al.*, 1994; Corcondilas *et al.*, 1964) and in situ (Marshall and Tandon, 1984) studies, rapid vasodilation must occur at exercise onset".

The results from this paper are subject to two criticisms: 1) our observations of greater effect of contraction on blood flow might be explained by a more effective emptying of venous volume with muscle contraction compared to cuff inflation, in other words a better muscle pump effect and not vasodilation is occurring with contractions 2) the mechanical compression of the forearm by cuff inflation actually causes a vasodilation and does not increase flow by emptying the veins

and reducing venous pressure. Figure 2.4 illustrates the results of a re-analysis of the data which contradict these criticisms and provide further support for our original interpretation. This figure shows the result of a beat-by-beat analysis of the hyperemia following a single cuff inflation vs. a single contraction with the arm positioned below heart level.

The rationale for interpretation of these data is as follows. According to the muscle pump hypothesis, the greatest effect of venous emptying on flow would be at the time immediately after the release of contraction or cuff inflation since this would be when the venous pressure is at its lowest. Therefore, if muscle contraction was more effective in emptying the veins than cuff inflation we would expect to observe a higher flow in the first beat following contraction compared with cuff inflation. Instead, we observed that the elevation in flow induced by contraction was not different from that due to the cuff, indicating that the cuff was a good analog of the mechanical effect of muscle contraction. Furthermore, if only the muscle pump contributed to the increase in flow during the first few seconds of exercise we would also expect that the highest flow would be observed during the beat immediately following release of contraction or cuff inflation and that flow would not increase during subsequent beats. In the cuff inflation condition this is indeed what happened, again suggesting the cuff has a purely mechanical effect. However following contraction, blood flow continued to rise after the first beat. This can only mean that a rapid vasodilation occurred with muscle contraction, and that it was detectable within 2 beats of the first contraction of exercise.

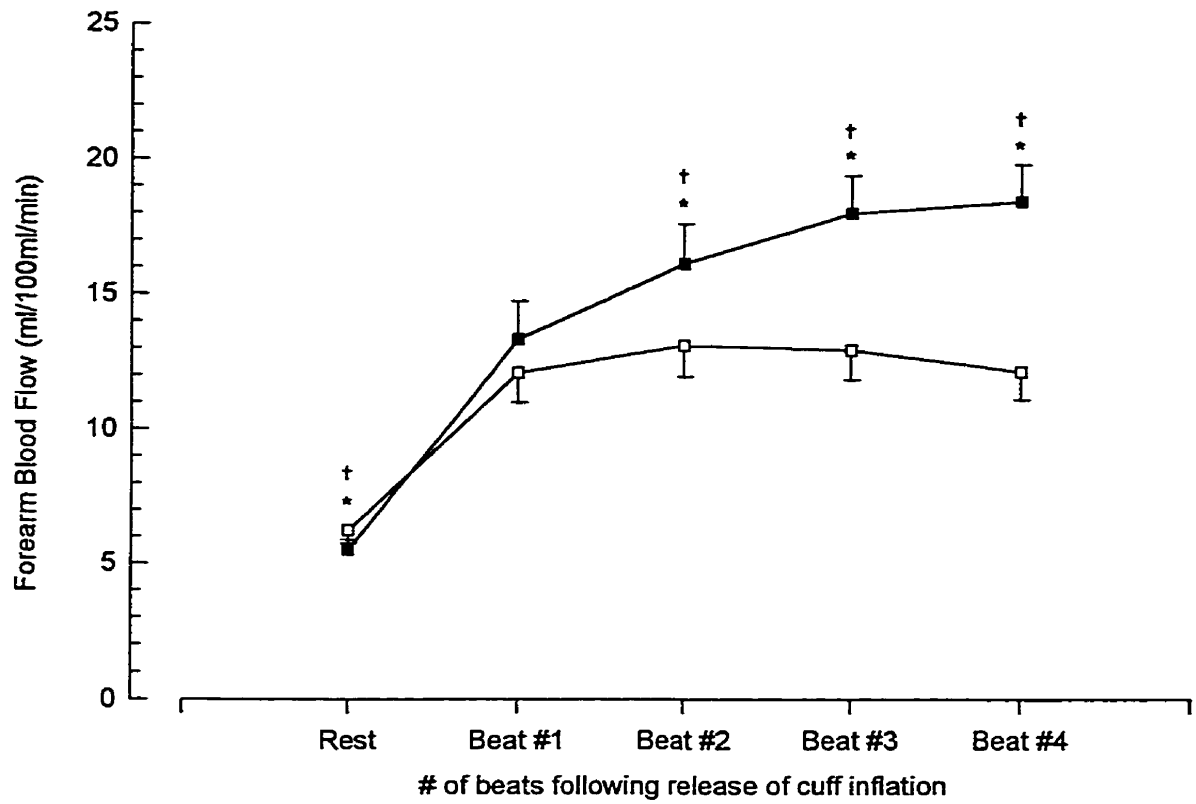

Figure 2.4 *Beat-by-beat forearm blood flow response to a single, 1-s forearm cuff inflation (cuff pressure 100 mmHg) (□) and a single, 1-s dynamic forearm contraction (8.6 kg, 5 cm displacement) (■). n=10. One-way repeated measures ANOVA at P<0.05. *significantly different from cuff inflation, †significantly different from beat #1.*

CHAPTER III

Reductions in venous volume and pressure with passive arm elevation: evidence for a venous volume and pressure contribution to local blood flow

ABSTRACT

We tested the hypothesis that reductions in venous pressure and volume can augment forearm blood flow (FBF) at rest and during exercise. 9 Subjects were seated with the right forearm supported in an arm rest below heart level. To temporarily empty the forearm veins, the subject was lowered for 4 s (acute) or 2 min (prolonged) and then raised again with the arm support rotating about a fixed axis. 3 conditions were performed during both acute and prolonged forearm elevation. Control: passive forearm elevation to empty forearm veins. Venous cuff: upper arm venous cuff inflation (30 mmHg) to prevent venous emptying. Forearm exercise: 2-s/ 2-s contraction relaxation schedule. Mean arterial pressure (MAP) at heart level and heart rate (HR) did not change with arm position or time. Reductions in forearm volume and venous pressure with arm elevation were prevented with venous cuff inflation. Lowering the forearm after 4 s increased FBF by 343% above baseline in control vs. 86% in the venous cuff condition. With prolonged forearm elevation a transient increase in FBF to 87% above baseline started within 6 s in the control but not the venous cuff condition. FBF increased by 149% vs. baseline upon forearm lowering after 2 min of forearm elevation in control vs. 118% in the venous cuff condition. In exercise, FBF decreased immediately by 48% on forearm elevation followed by a partial recovery. Forearm lowering after 4 s or 2 min of elevation during exercise resulted in a transient overshoot of FBF by 44% and 42% respectively. These results suggest that venous emptying on forearm elevation evokes vasodilation that might be a consequence of the veno-arteriolar reflex. Both this transient vasodilation and the increased arterial to venous pressure gradient can contribute to the transient increase in FBF on lowering.

Keywords: Doppler ultrasound, veins, vasodilation, veno-arteriolar reflex

INTRODUCTION

Blood flow through a vascular bed is dependent on the upstream-downstream pressure gradient (ΔP) and the vascular conductance (VC). Evidence exists that venous pressure might 1) act as the effective downstream pressure and therefore impact ΔP, and 2) affect arterial vascular tone, thereby modulating VC. Folkow et al. (1971) observed that when humans were tilted from the supine to the upright position there was an increase in calf blood flow during exhausting calf plantar flexion exercise. In contrast, they found that post-exercise hyperemia was not facilitated, possibly as a consequence of rapid venous refilling (Folkow *et al.*, 1971). They proposed that muscle contraction emptied the veins and increased the pressure gradient across the capillary bed of the muscle. A reduction in venous pressure immediately after a muscle contraction has been documented (Pollack and Wood, 1949; Stick *et al.*, 1992; Folkow *et al.*, 1970). This evidence infers that venous pressure acts as the effective downstream pressure for arterial inflow. Recently, we have demonstrated that mechanical compression to empty forearm veins results in an elevation in forearm blood flow below but not above heart level (Tschakovsky *et al.*, 1996), supporting the contention that a reduction in venous pressure enhances arterial inflow. However, other investigators have provided evidence that venous pressure does not act as the downstream pressure for arterial inflow (Naamani *et al.*, 1995; Magder, 1995). For example, in isolated in situ dog muscle that was maximally vasodilated, Naamani et al. (1995) did not observe an increase in blood flow with contractions, nor were they able to alter flow by manipulating venous pressure in such a preparation. This is consistent with the phenomenon of a zero flow pressure intercept observed in a number of other studies using a similar in situ preparation, in which zero arterial inflow occurs at arterial pressures that are well above venous pressure (Jackman and Green, 1990;

Shrier and Magder, 1995; Permutt and Riley, 1963). It must be mentioned here that these studies were all conducted on in situ animal muscle preparations whereas evidence supporting the enhancement of arterial inflow with venous emptying comes from in vivo studies of animals and humans. This suggests caution in the interpretation and application of vascular responses from in situ animal preparations to the in vivo condition.

Evidence for an effect of venous pressure on arterial VC was first provided by Gaskell and Burton (1953), who demonstrated a postural reflex arising from limb veins which resulted in arterial vasoconstriction when the veins filled with blood. Since then, numerous studies have demonstrated the existence of this "veno-arteriolar" reflex in subcutaneous (Henriksen, 1991; Vissing *et al.*, 1997) and muscle tissue (Henriksen and Sejrsen, 1977; Henriksen *et al.*, 1983), demonstrating a reduction in blood flow when a limb is lowered into the dependent position. This reflex appears to trigger a vasoconstriction once a threshold venous pressure of ~25 mmHg is reached (Henriksen, 1991). Evidence has also been presented suggesting that muscle contraction-induced emptying of the veins prevents this vasoconstriction from occurring (Nielsen, 1982). However, a release of this vasoconstriction when the veins are emptied as would occur when a limb is moved from a dependent position to above heart level has not been clearly established. In addition, the time course of the in vivo blood flow responses to alterations in venous pressure are unknown due to the poor time resolution of methods such as ^{133}Xe clearance commonly used to investigate these responses (Nielsen, 1982; Henriksen *et al.*, 1983; Henriksen and Sejrsen, 1977).

With this information as a background, we tested the hypothesis that reductions in forearm venous volume (and therefore pressure) elevate forearm blood flow (FBF) at rest and exercise. The application of Doppler ultrasound allowed us to achieve a beat by beat time resolution which

could provide information on the adaptive characteristics of the blood flow responses not attainable with conventional in vivo methods of measuring limb blood flow such as strain gauge plethysmography or ^{133}Xe clearance. Our approach was to elevate the forearm supported in an armrest from below to above heart level in order to empty the venous volume. Then, after acute (4-s) or prolonged (2-min) elevation, the arm was lowered to re-establish local arterial perfusion pressure under conditions of reduced venous volume and pressure.

METHODS

Subjects

9 healthy female subjects participated in this study (age 22.8 ± 1.2 yrs, height 167.4 ± 2.3 cm, weight 59.4 ± 2.1 kg) (mean ± SE) and gave written consent on a form approved by the Office of Human Research of the University after receiving full written and verbal details of the experimental protocol and any potential risks involved.

Experimental Apparatus

In order to achieve changes in resting forearm position relative to heart level, subjects sat upright in a chair with their right arm supported in an arm rest. The arm rest supported the right forearm at the wrist and from just distal of the elbow to approximately half way up the length of the upper arm. The chair could be raised and lowered via a pulley system, in effect raising and lowering the heart relative to the arm, since the arm rest remained at the same height. Raising and lowering of the subject resulted in an average mid-forearm level of 18.9 ± 2.0 cm below heart level (arm below) and 25.0 ± 0.5 cm above heart level (arm above). For the exercise, subjects gripped a small hand-held device (Gripmaster IMC Prod Corp., Hicksville, NY) that had a range of compression of 3 cm and required a force equivalent to lifting a 6.5 kg weight.

Experimental Protocol

On arrival in the laboratory, ECG electrodes (CM_5 placement) were applied to the skin and a 20-gauge catheter inserted in a retrograde direction to venous flow in an antecubital vein (5 of the 9 subjects). Subjects were then seated in the chair and the arm rest position was adjusted to correspond with the range of elevation of the chair. This resulted in the average mid forearm levels relative to the heart as just mentioned. The chair was then lifted into the arm below

condition. This served as the control or baseline condition. In those subjects in which venous pressure was being measured, the catheter was connected via a short length of sterile heparinized saline filled tubing to a pressure transducer (Gould P23 Db series, Gould Inc., Oxnard Ca.). The catheter was also connected to a vertical tube used for a two-point calibration of the pressure transducer in cm H_2O. This was then filled with sterile heparinized saline solution. The transducer was affixed to the arm rest at the level of the catheter tip. A pneumatic finger blood pressure cuff (Ohmeda 2300, Finapres, Lakewood, CO) was placed around the middle finger of the left hand, and the subjects held this finger at right mid forearm level as they sat for 2 min first in the arm below and the left arm was supported in a sling so that the finger blood pressure cuff was at mid-sternum level (~ right atrial level) for the duration of the experiment. It was not possible to make simultaneous measurements of brachial artery diameter and velocity due to technical limitations. Therefore, brachial artery diameter (echo Doppler, Toshiba model SSH-140A, Tochigi-Ken, Japan) was measured during separate trials in both the acute and prolonged forearm elevation protocol (Figure 3.1) to obtain the diameter response for these experimental conditions used in the calculation of forearm blood flow.

Subjects began with the forearm below heart level for all experimental conditions. Figure 3.1 depicts the two protocols for changing forearm position. In the acute forearm elevation protocol, the forearm began in the below heart position for 10 s prior to lowering the subject. At time = 0-s, the chair was lowered over a 2-s period such that at 2 s the forearm was in the above heart position. At 6 s the chair was raised over a 2-s period so that the forearm was once again in the below heart position. Data collection continued until 140 s. In the prolonged arm elevation protocol, the forearm also began in the below heart position for 10 s followed by the same chair

lowering over a 2-s period. The forearm then remained in the above heart position for 2 min and was then lowered over a 2-s period. Data collection continued until 290 s. For the exercise protocol, the timing of the lowering of the forearm was delayed by 2 s so that it coincided with a 2-s contraction and therefore relaxation began as the forearm assumed the below heart position. All subjects performed at least 3 trials in each condition.

Within each of these two protocols, three different conditions were tested. Condition 1 (control): the right forearm was at rest at all times during raising and lowering. Condition 2 (venous cuff): the right forearm was at rest at all times during raising and lowering. A venous occlusion cuff around the upper arm was rapidly (<0.5 s) inflated to ~ 30 mmHg (pilot work indicated this to be the required pressure to maintain arm volume) immediately prior to the forearm moving into the above heart position, and rapidly (< 0.5 s) deflated immediately after the forearm was again in the below heart level position. Condition 3 (exercise): continuous forearm exercise was performed (2-s/ 2-s contraction/relaxation cycle timed so that relaxation occurred as the forearm achieved its new position relative to heart level) was performed. Subjects began exercise in the arm below heart position for 4 min to achieve a steady state blood flow prior to the acute and prolonged forearm elevation protocols.

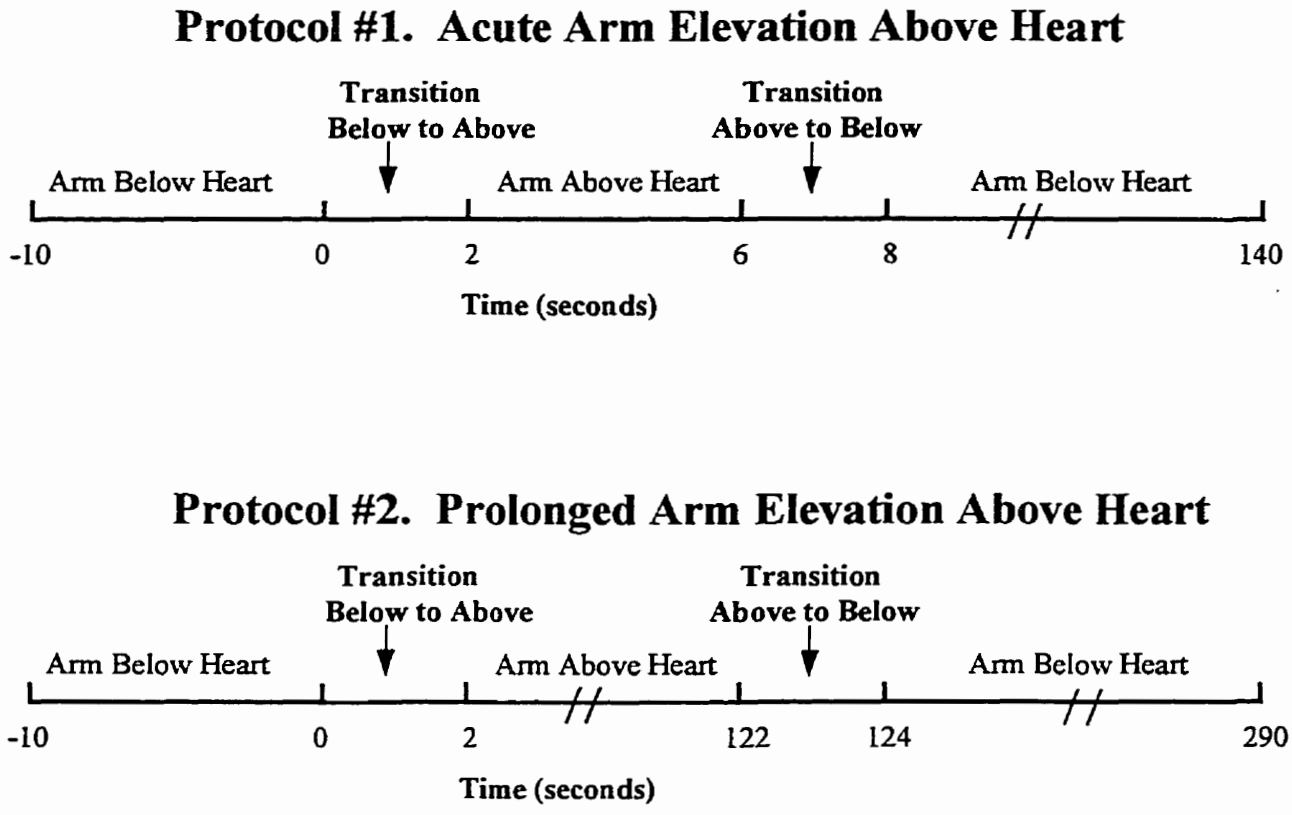

Figure 3.1 *Schematic illustration of the timing of arm position above and below heart level during the acute and the prolonged arm elevation protocols. This was the timing for both the control condition and the venous congestion cuff condition. For exercise in the acute arm elevation protocol, lowering of the arm occurred at 8 - 10 s and for exercise in the prolonged arm elevation protocol lowering of the arm occurred at 124 - 126 s. This was done so that the transition coincided with a 2-s contraction and therefore relaxation began immediately as the arm assumed the below heart position.*

Data Acquisition

Heart rate (HR) and mean arterial pressure (MAP) were measured beat by beat. MAP was measured at heart level using a photoplethysmograph finger blood pressure cuff (Ohmeda 2300, Finapres, Lakewood, CO) on the middle finger of the left hand.

Forearm blood flow (FBF) was obtained beat by beat as the product of brachial artery mean blood velocity (MBV) and arterial cross sectional area:

$$FBF\ (ml/min) = MBV\ (cm/s) \bullet 60\ s/min \bullet \pi(\text{brachial artery diameter (cm)}/2)^2$$

Brachial artery blood velocity was measured with a 4-MHz pulsed Doppler ultrasound probe (Multigon Industries, model 500V, Mt. Vernon, NY) which was fixed to the skin over the brachial artery at the level of the antecubital fossa of the right elbow (Tschakovsky *et al.*, 1995). With this placement and arm position, probe insonation angle relative to the skin is 45° and the brachial artery is approximately parallel with the skin. Arterial cross-sectional area was measured by a separate, linear 7.5 MHz echo Doppler ultrasound probe operating in B mode (Toshiba model SSH-140A, Tochigi-Ken, Japan) during an acute forearm elevation and a prolonged forearm elevation trial prior to the experimental trials, since it was not possible to obtain simultaneous velocity and artery diameter measurements. Imaged data were saved on video tape for subsequent analysis. There was no difference in diameter between the two arm positions or over time, therefore the diameter values used to calculate brachial artery blood flow were the average of 10 separate measures of diameter over the duration of each of the acute and prolonged arm elevation protocols. Diameter measurements at these times consisted of the average of 3 separate caliper measures of a frozen screen image of the brachial artery during diastole. All measurements were performed by the same operator.

To express FBF in ml/100ml/min forearm volume was measured in each subject prior to the experiment in the dependent position via water displacement. Forearm volume averaged 714 ± 29 ml (mean ± SE).

Forearm Volume

A mercury in silastic rubber strain gauge (Hokanson EC-4 plethysmograph, D.E. Hokanson Inc.) was placed around the right forearm at the point of largest circumference. When the arm was in the below heart position, the gauge was reset to 0, indicating baseline volume. Changes in arm volume with altered limb position could then be followed and expressed relative to baseline. Calibration of the strain gauge was performed with an internally generated voltage equivalent to a 1% change in cylinder volume.

Brachial artery MBV, MAP, HR, forearm volume and venous pressure were all collected at 100 Hz on the same dedicated computer.

Statistical Analysis

Specific hypothesis testing comparing responses within a condition across changes in arm position was performed with one way repeated measures ANOVA and further multiple comparisons were performed with a Student-Newman Keuls post hoc test when ANOVA indicated significant differences existed across time within a condition. Comparisons between conditions at specific times during the arm elevation and lowering were performed with one way repeated measures ANOVA. The level of significance for ANOVA was set at $P<0.05$. All data are presented as means ± SE.

RESULTS

There were no changes in either HR or MAP with time in any of the experimental conditions. Therefore changes in FBF could be interpreted with respect to changes in vascular tone or changes in the hydrostatic component of the local pressure gradient as the forearm was moved relative to heart level. Continuous measures of brachial artery diameter in the control condition during both the acute and prolonged arm elevation protocols showed no effect on brachial artery diameter due to arm position or time.

Acute forearm elevation from below to above heart level

Figure 3.2 provides 1-s interpolated FBF (Doppler), Δ Arm Volume from the baseline forearm below heart position (strain gauge plethysmography) and venous pressures in an antecubital vein at the elbow (n=4 for control, n=3 for venous cuff) in the acute forearm elevation protocol. Data are not shown for the transitions because of motion artifacts in some subjects.

In both the control condition where the veins were allowed to drain upon forearm elevation and the venous cuff condition where venous volume was maintained during forearm elevation, FBF was not different from baseline below heart during the 4-s forearm elevation. When the forearm was then lowered to the below heart level, a transient hyperemia was observed in both conditions. However, this hyperemia was minor in the venous cuff condition (peak vs. baseline: 4.1 ± 0.5 vs. 2.2 ± 0.1 ml/100ml/min ● SE, P=0.003), compared to control (peak vs. baseline: 10.2 ± 1.4 vs. 2.3 ± 0.1, P=0.0002). The difference between the peak hyperemia in control vs. cuff inflation was highly significant (P=0.0008). In control, the forearm volume decreased markedly on arm elevation and recovered slowly on return to the arm below heart position. In contrast, the forearm volume appeared to increase slightly during elevation with the venous cuff

inflated, likely due to a redistribution of volume in the arm up position, but returned to baseline immediately on lowering the arm. This indicated that the venous cuff was successful in maintaining total forearm volume during elevation. The changes in venous pressure paralleled those in forearm volume. When these responses are expressed as % change from baseline, the faster restoration of venous pressure compared to volume upon forearm lowering in control becomes evident (Figure 3.4, panel A).

Prolonged forearm elevation from below to above heart level

Similar to acute forearm elevation, blood flow immediately upon prolonged forearm elevation was not different from baseline in the control and venous cuff condition (Figure 3.3). However, within 5 s of forearm elevation, FBF had increased compared to baseline in control, peaking by 8 s (3.9 ± 0.4 vs. 2.1 ± 0.1, P=.0004). Thereafter, FBF fell over the next few seconds but stabilized at a level that was still significantly elevated vs. baseline (40-50 s average: 2.6 ± 0.2 vs. baseline: 2.1 ±0.1, P=0.008). In contrast, when venous volume upon forearm elevation was maintained with the venous cuff, no transient hyperemia was observed. However, FBF did increase slightly but significantly over time such that it soon matched the FBF observed after the transient hyperemia in the control condition (40-50s average venous cuff: 2.6 ± 0.2). Thereafter, flow continued to increase slightly in the control condition (significantly elevated 110-120 s average: 3.1 ± 0.2 vs. 30-40 s average: 2.6 ±0.2, P<0.05) but did not change in the venous cuff condition. When the forearm was lowered to the below heart position after the prolonged period of elevation, a similar transient hyperemia was observed in the control and venous cuff condition (5.3 ± 0.5 and 4.8 ± 0.5).

As in the acute forearm elevation condition, the venous cuff was successful in maintaining

total forearm volume during the 2 min of forearm elevation as indicated by the immediate return of forearm volume and venous pressure to baseline below heart levels upon forearm lowering and cuff deflation. In control, an initial rapid fall in forearm volume and venous pressure was followed by a second slow, progressive decrease over the 2 min of forearm elevation. Upon lowering the forearm, venous pressure rapidly returned to baseline below heart levels while forearm volume increased much more slowly. Again, the difference in the time course of forearm volume vs. pressure restoration can be appreciated when expressed as % baseline in Figure 3.4.

Acute and prolonged forearm elevation: effect during exercise

Figure 3.5 shows the FBF (panel A, n=8) in the 2-s relaxation phases during 2-s/2-s contraction/relaxation forearm exercise. A substantial reduction in FBF during exercise occurred immediately upon forearm elevation in both the acute and prolonged forearm elevation conditions. However, by the second relaxation phase blood flow had partially recovered. When the forearm was then lowered below heart level, a transient overshoot in FBF above baseline was maintained for the next 2 relaxation phases. When the forearm remained above heart level, FBF showed no further recovery from that during the initial few seconds of forearm elevation and maintained a steady state below the baseline below heart level observed prior to forearm elevation. When the forearm was finally lowered, a similar transient overshoot to that observed during acute forearm elevation occurred followed by a rapid down regulation of FBF. FBF then fluctuated at or significantly above baseline.

Instantaneous mean blood velocity profile

Figures 3.6 and 3.7 provide beat by beat instantaneous brachial artery mean blood flow velocity waveform data from a single subject. Resting blood flow was characterized by a brief

systolic inflow pulse and a small retrograde pulse followed by zero inflow during diastole. In Figure 3.6 it can be appreciated that immediately upon forearm elevation, the systolic peak velocity was elevated, but at the same time there was increased retrograde flow such that total inflow was not altered from the below heart condition. This occurred regardless of whether venous drainage was allowed or not. When the forearm was lowered after 4 s of elevation where venous drainage had occurred, significant diastolic blood flow was observed. In contrast, when no venous drainage occurred, no diastolic blood flow was observed, although initially the small retrograde flow was not present.

In exercise, the relaxation phase was where most blood flow occurred and was characterized by considerable diastolic flow (Figure 3.7). Immediately upon elevating the forearm both systolic and diastolic blood flow were reduced, but both showed a recovery by the second relaxation phase. When the forearm was then lowered (Figure 3.7, panel A), there was a large increase in both systolic and diastolic blood flow in excess of that observed before the arm had been raised. When the arm was maintained in the above heart position, the partial recovery of systolic and diastolic flow was maintained.

Venous Pressure Volume Relationships

Figure 3.4 indicates that when the forearm was elevated above heart level, forearm volume and venous pressure changes occurred at a similar rate. However when the forearm returned to below heart level, venous pressure increased more rapidly than volume, indicating a hysteresis. This hysteresis in the venous pressure-forearm volume relationship when the veins were emptying vs. filling is illustrated clearly in Figure 3.8. Peak forearm volume and venous pressure occurred with the arm in the below heart position. When the arm was elevated above heart level, venous

drainage occurred and venous pressure and volume dropped rapidly at first and then more slowly if the arm remained elevated. When the arm was again lowered, the pressure volume relationship shifted such that large changes in pressure with relatively small changes in volume occurred early during refilling. Continued increases in forearm volume occurred when venous pressure was essentially back to initial below heart levels.

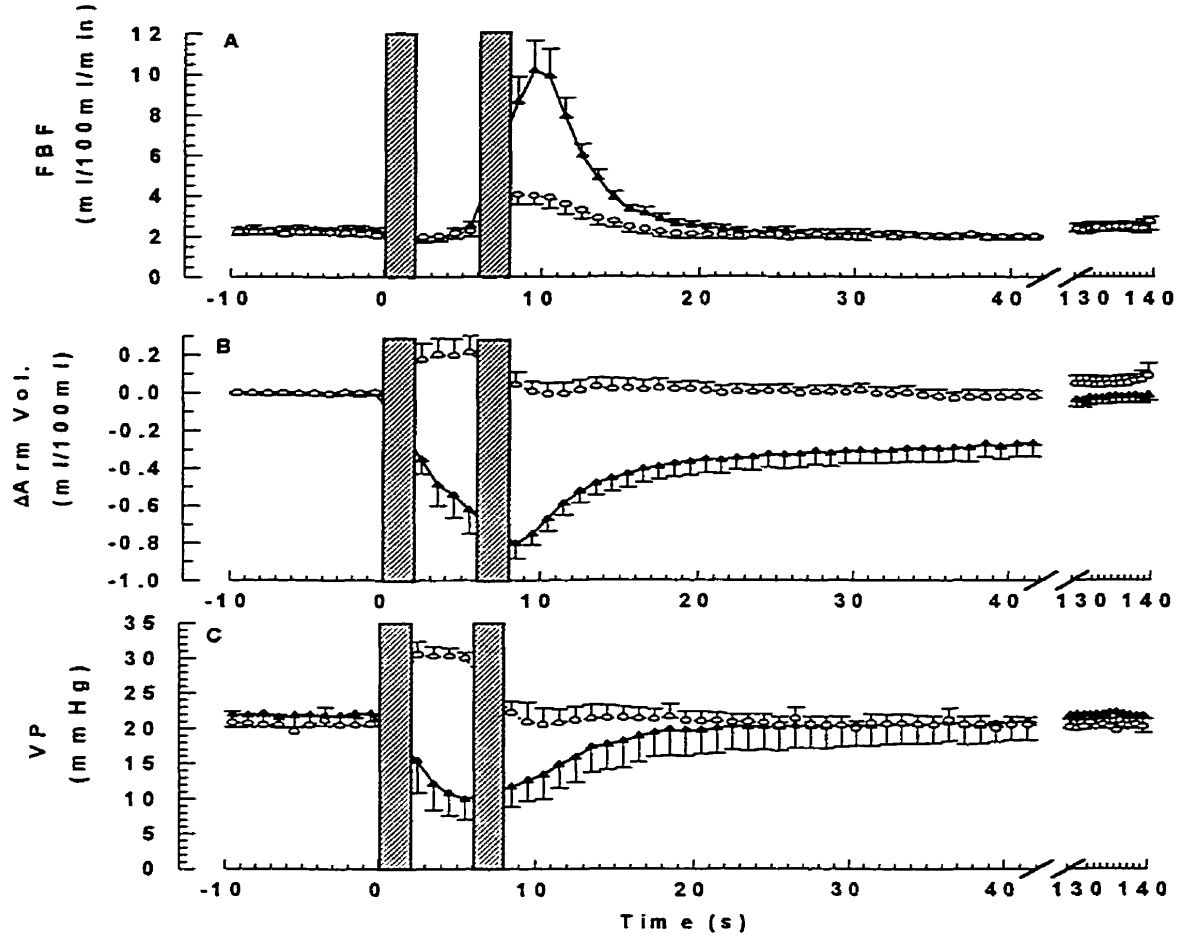

Figure 3.2 *Acute forearm elevation protocol: 1 s interpolated forearm blood flow (FBF) (A), change in forearm volume from below heart baseline (Δ Arm Vol.) (B), and venous pressure (VP) (C). Hatched boxes indicate 2 s transitions between forearm positions. The forearm began below heart level (baseline, -10-0 s). Control where venous drainage occurred upon forearm elevation (—▲—), Venous cuff inflated to maintain forearm volume from 0-8 s (····○ ····). FBF was significantly elevated vs. baseline for 7 s following forearm lowering in control and 5 s in venous cuff. Forearm volume did not returned to baseline levels until 130-140 s. Venous pressure was no longer significantly lower than baseline in control by 25 s (all $P<0.05$, $n=9$ except VP where $n=4$ control, $n=3$ venous cuff).*

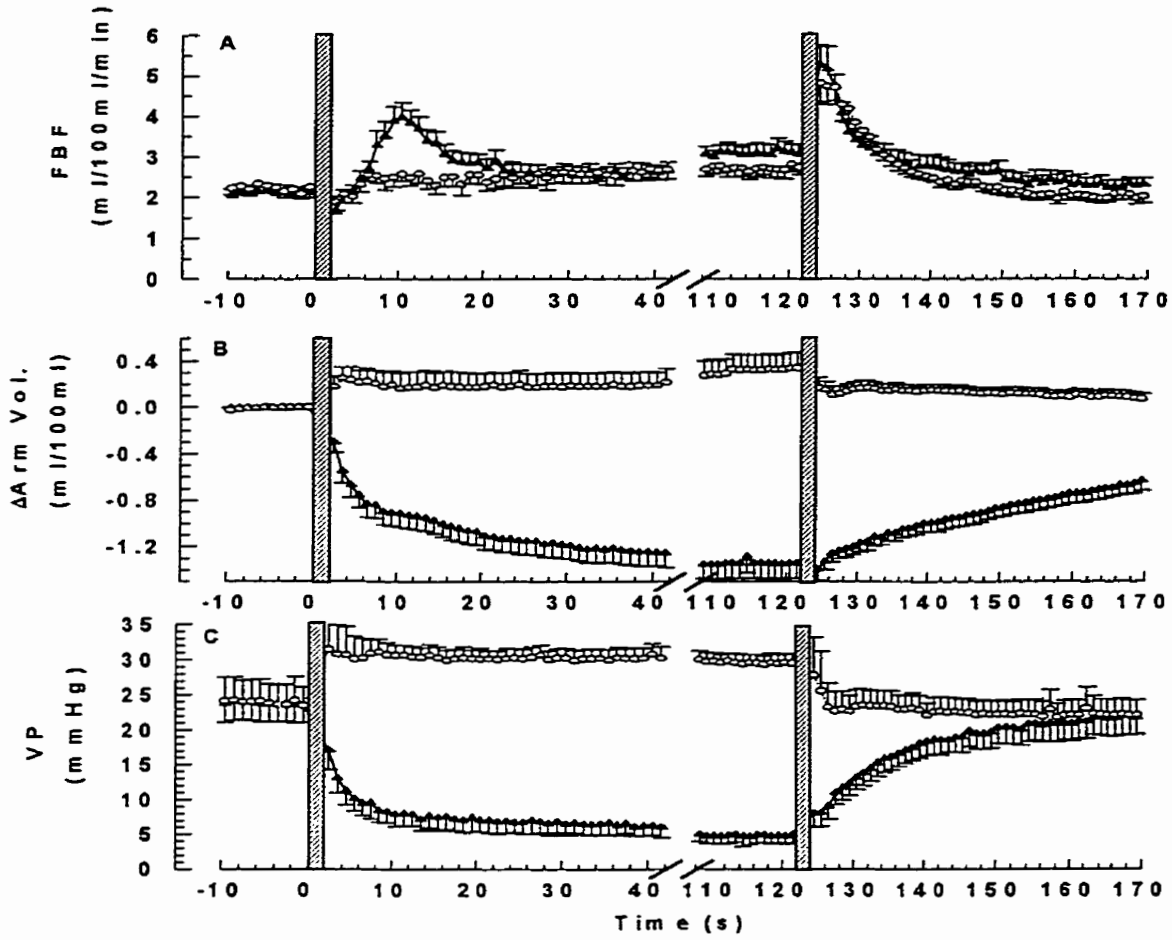

Figure 3.3 *Prolonged forearm elevation protocol. 1 s interpolated forearm blood flow (FBF) (A), change in forearm volume from below heart baseline (Δ Arm Vol.) (B), and venous pressure (VP) (C). Hatched boxes indicate 2 s transitions between forearm positions. The forearm began below heart level (baseline, 0-10 s). Control, where venous drainage occurred upon forearm elevation (—▲—), Venous cuff inflated to maintain forearm volume 10-124 s (····○ ····). FBF was significantly elevated vs. baseline by 6 s of forearm elevation in control and by 30-40 s in venous cuff condition. Upon arm lowering FBF remained significantly above baseline for 13 s. Forearm volume did not return to baseline levels until 280-290 s (not shown) (all $P<0.05$, $n=9$ except VP where $n=5$ control, $n=3$ venous cuff).*

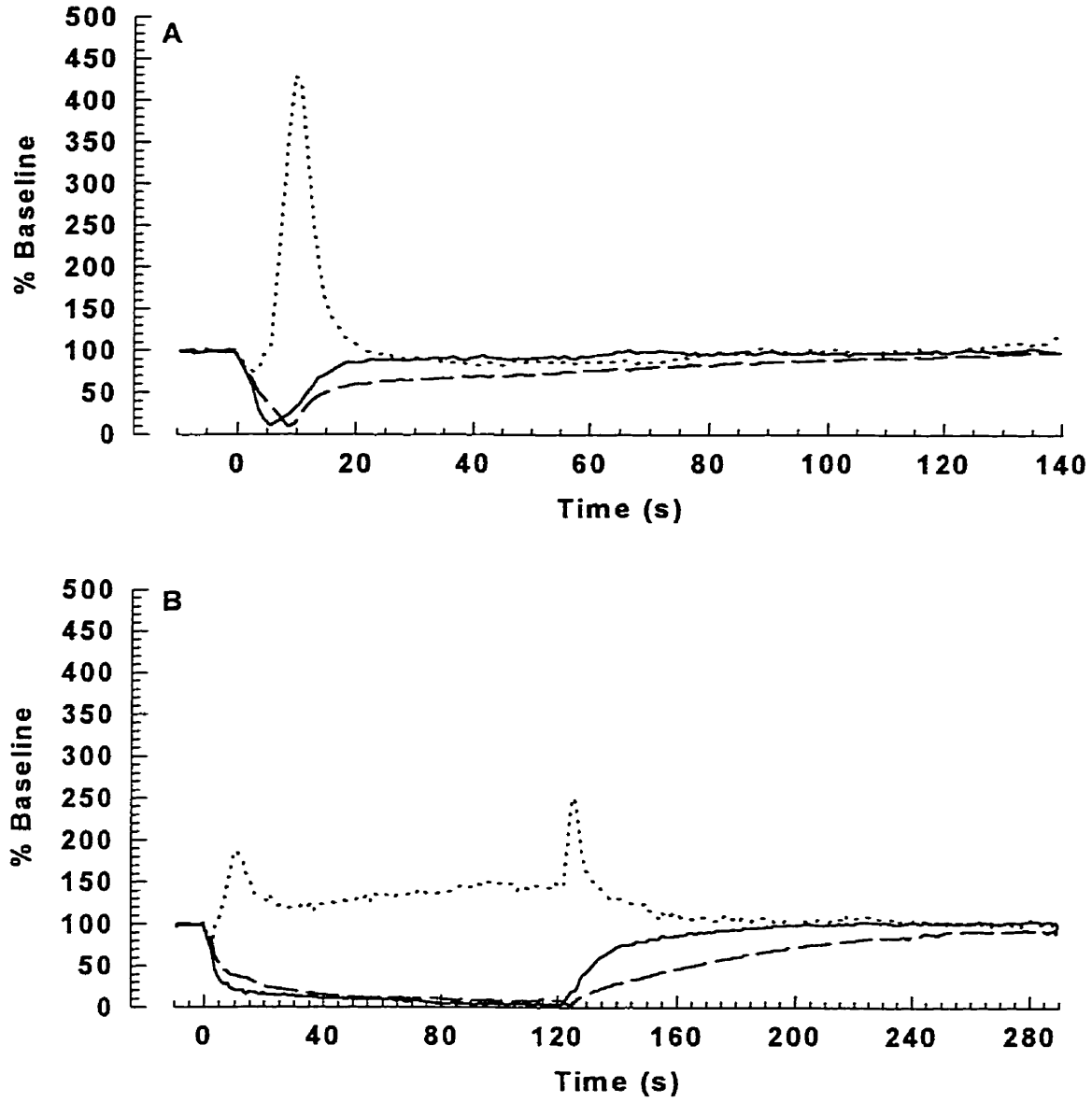

Figure 3.4 *Acute forearm elevation protocol (A) and prolonged forearm elevation protocol (B) for the control condition where the veins were allowed to drain upon forearm elevation: % of forearm below heart baseline for forearm blood flow (·····), forearm volume (— —) and venous pressure (——). For venous pressure and forearm volume, 0 % represents the lowest value achieved during forearm elevation.*

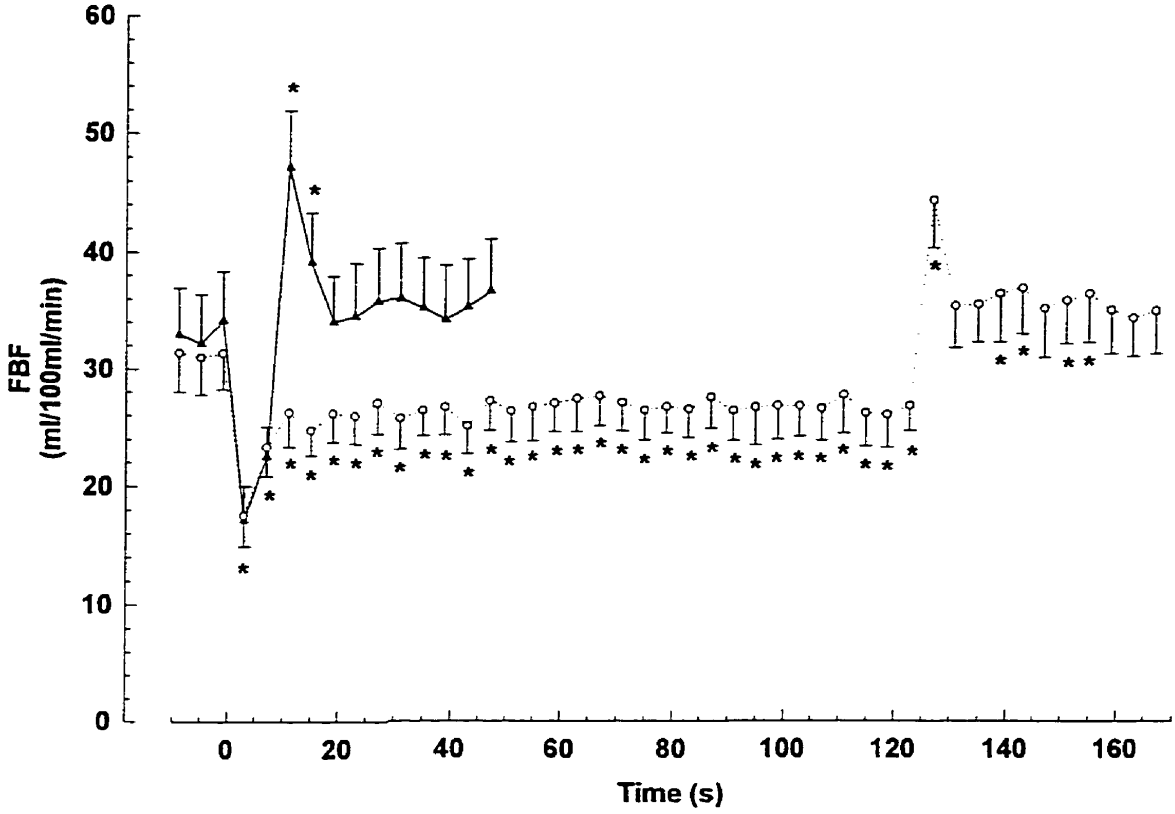

Figure 3.5 *Relaxation phase blood flow in forearm exercise during the 2-s relaxation phases between 2-s contractions. Acute forearm elevation(—▲—), prolonged forearm elevation (····O····). Forearm began below heart level (-10-0 s) and was elevated between 0-2 s. In the acute arm elevation protocol, the forearm was then lowered between 8-10 s, while in the prolonged arm elevation protocol lowering occurred between 124-126 s. *Significantly different from baseline (P<0.05).*

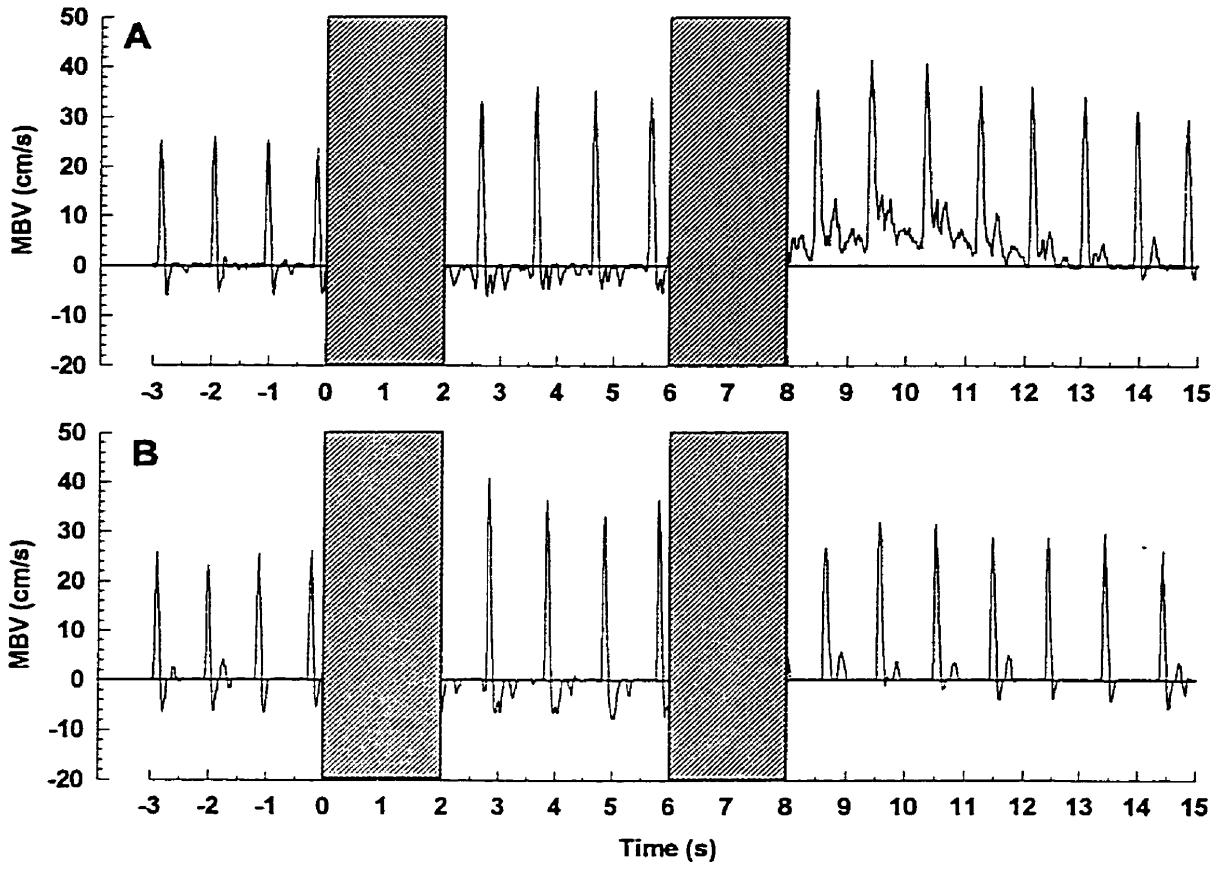

Figure 3.6 *Example from a single subject of beat by beat arterial inflow blood velocity waveforms in response to acute forearm elevation when venous drainage was allowed (control, A) and when it was prevented (venous cuff, B). Hatched bars indicate forearm position transition periods (see Figure 3.1).*

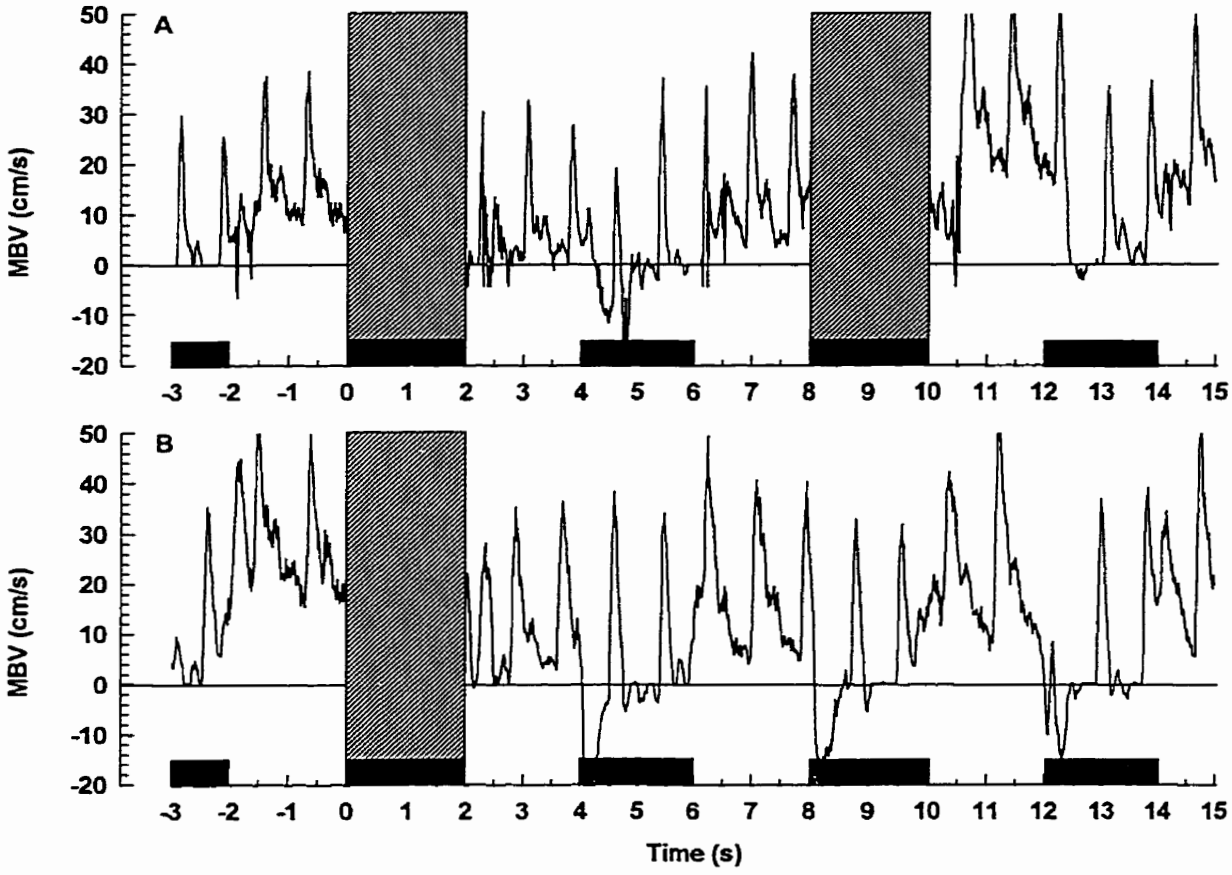

Figure 3.7 *Example from a single subject of beat by beat arterial inflow blood velocity waveforms during exercise in response to acute forearm elevation (A) and maintained forearm elevation (B). Hatched bars indicate forearm position transition period (see Figure 3.1) Solid bars indicate when contractions occurred.*

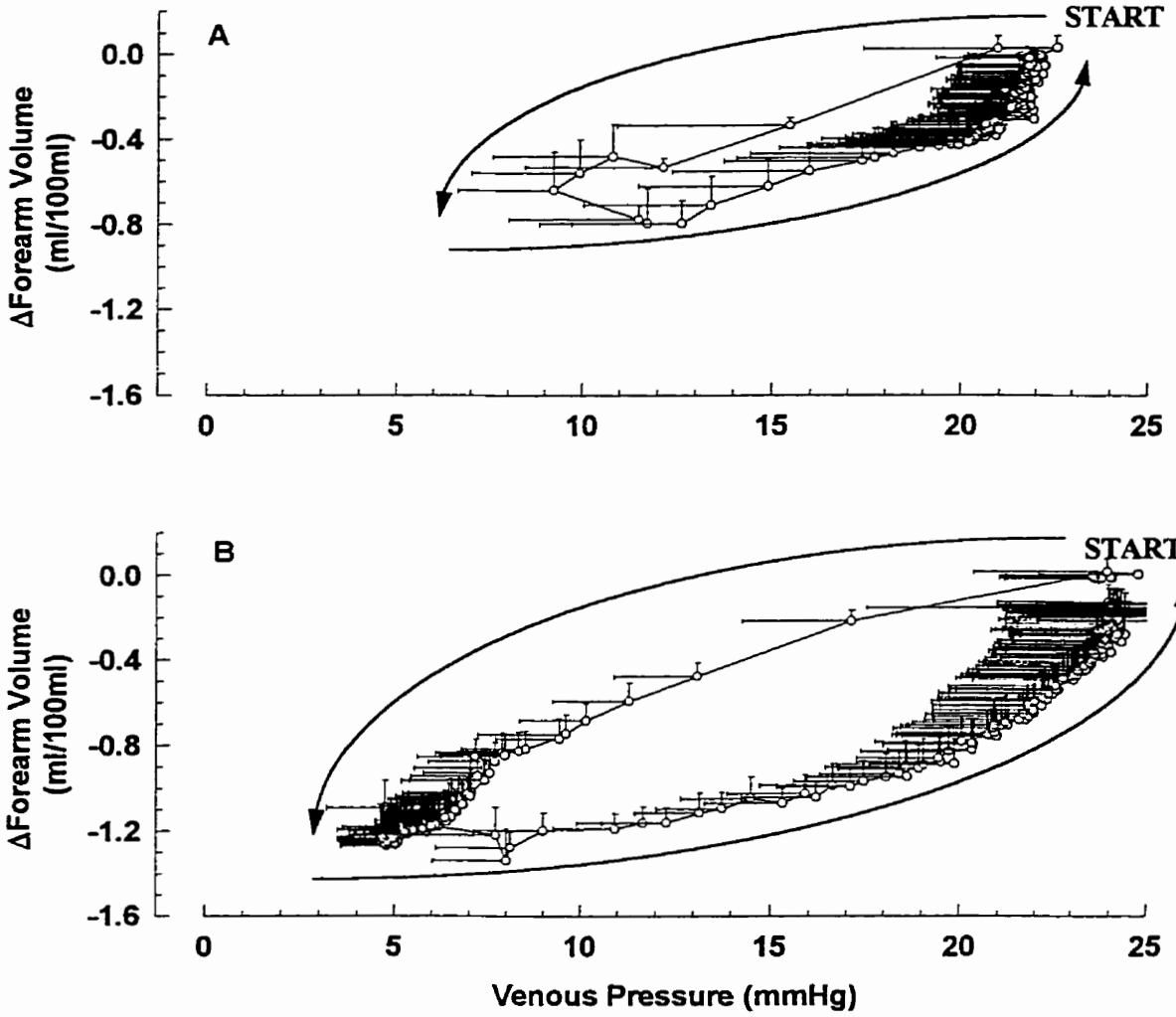

Figure 3.8 *Change in forearm volume vs. venous pressure over time during acute arm elevation (A) and prolonged arm elevation (B) in the control condition. START indicates time = -10 s as in Figure 3.2 and 3.3. Each data point represents 1-s interval ± SE, n=4. Arrows indicate the direction of time. Forearm began in the arm below heart position at START and at 10 s was elevated above heart level for 4 s in A and 2 min in B. Hysteresis is evident in both conditions but is more pronounced in B.*

DISCUSSION

This study sought to determine whether reductions in venous pressure can increase vascular conductance (VC) and/or contribute to changes in the effective upstream-downstream pressure gradient (ΔP), thereby elevating forearm blood flow (FBF). It has been demonstrated by others that movement of a limb into the dependent position results in a vasoconstriction that is triggered by the filling of the veins (Henriksen and Sejrsen, 1977; Henriksen *et al.*, 1983; Henriksen, 1991; Vissing *et al.*, 1997). If the muscle pump is activated to prevent venous filling on assuming dependency, this vasoconstriction does not appear to occur (Nielsen, 1982). Based on this we hypothesized that emptying of the veins by arm elevation would remove a veno-arteriolar reflex mediated vasoconstriction and result in an increase in forearm VC. The observation of a transient increase in FBF upon arm elevation that could be abolished by preventing venous emptying supports this hypothesis. In addition, this study is the first to document the time course of changes in VC due to the withdrawal of this veno-arteriolar vasoconstriction and indicates that there is a transient overshoot in vasodilation.

Whether venous pressure acts as the effective downstream pressure that establishes the pressure gradient to determine arterial inflow is not clear. We (Tschakovsky *et al.*, 1996) and others (Sheriff *et al.*, 1993; Folkow *et al.*, 1971) have provided evidence supporting the muscle pump hypothesis which states that relaxation after muscle contractions can reduce venous pressure by squeezing blood out of the veins and that this increase in the local perfusion pressure gradient results in an increase in blood flow. However, others using in situ animal muscle preparations have observed that manipulations of venous pressure did not alter arterial inflow (Jackman and Green, 1990; Shrier and Magder, 1995; Permutt and Riley, 1963; Naamani *et al.*,

1995; Magder, 1995). We observed a substantial hyperemia upon lowering the arm after 4 s of elevation when the veins were allowed to drain. The estimated increase in VC due to a removal of veno-arteriolar vasoconstriction that may have occurred at the time of arm lowering could not account for all of the hyperemia on acute arm lowering. This suggests a contribution of the increased effective upstream-downstream pressure gradient for arterial inflow due to reductions in venous pressure with arm elevation, supporting the hypothesis that venous pressure might act as the effective downstream pressure.

Critique of the Experimental Model

This study was designed to determine in vivo the effect of reductions in venous pressure on arterial inflow. To this end, knowledge of venous pressure, specifically post capillary venule pressure is desirable. However, technical limitations preclude the dynamic measurement of venule pressure in the in vivo condition. Our measures of venous pressure were obtained in an antecubital vein in a limited number of subjects by inserting a 1.5 inch catheter in a retrograde manner at the elbow. This meant that we were determining pressure in a segment of vein that was external to the deep tissue. Normally, limb elevation above heart level results in venous pressures of 1-3 mmHg in the collecting veins (Nielsen, 1991). We observed venous pressures that were ~10 mmHg within 4 s of arm elevation and ~5 mmHg after 2 min of arm elevation, indicating that venous drainage may have been slightly impeded. This was likely due to a compression of veins on the underside of the upper arm by the arm rest support, which meant that a venous hydrostatic column may have been contributing to pressure at the site of the catheter. However, given that the arm was angled hand up, it is likely that venule pressure in the forearm and hand was lower than that at the measurement site. Therefore it can be assumed with confidence that arm elevation

did result in substantial effects on venule volume and pressure.

In this experiment, subjects were seated upright in a chair with their right arm supported in an arm rest. Subjects sat in a chair that could be elevated or lowered on a vertical track by means of a pulley system. The pivot point for the arm rest was at the level of the elbow so that arterial and venous pressures were not uniform along the length of the forearm. When the venous congestion cuff on the upper arm was inflated just prior to arm elevation, this angling of the arm meant the arm was analogous to a partially filled bottle. When the bottle is tipped, the distribution of volume changes in accordance with gravity, although the total volume in the bottle remains the same. Consistent with such an effect during experiments where the venous congestion cuff was inflated were observations of elevated forearm circumference just distal to the elbow as measured by the strain gauge and increased venous pressure as measured at the site of venous catheterization upon arm elevation. This effect meant that in the venous congestion cuff experiments there were likely acute alterations in venule pressure that varied depending on location in the forearm. Therefore, this condition did not necessarily alter the local arterial-venous pressure gradient in proportion to hydrostatic effects on the arterial side in the first few seconds of arm elevation. Nevertheless, as was evidenced by the immediate return to baseline venous pressure at the elbow and forearm circumference at the site of strain gauge measurement, the congestion cuff was entirely successful in maintaining total forearm venous volume during arm elevation.

Blood Flow Immediately Upon Arm Elevation

The beat by beat time resolution of Doppler ultrasound measures of FBF allowed us to assess the response immediately upon elevation of the arm above heart level. FBF should be dependent

upon the local vascular conductance (VC) and the upstream-downstream pressure gradient. When the arm was elevated, reductions in local arterial and venous pressure proportional to changes in the hydrostatic column should have occurred. In addition, rapid reductions in forearm venous volume should have occurred as blood drained towards the heart. As mentioned, a venous congestion cuff was used to prevent a change in total forearm volume with arm elevation in some experiments, however the acute effect on local venule volume and pressure may have been similar to that when veins drained freely due to the angled arm position which likely rapidly redistributed volume from the venules to the large collecting veins.

We observed no reduction in arterial inflow at rest immediately upon arm elevation, regardless of whether venous drainage was allowed or not. Given that arterial pressure at arm level was reduced, we would have expected a transient reduction in blood flow, which should have been even greater when venous volume was not allowed to drain. Two possible explanations exist to explain a lack of effect of maintained venous congestion. First, the fact that the arm was angled up means that, although total venous volume was preserved with the venous cuff, the acute distribution of that blood may have been out of the venules, effectively decreasing the pressure there. An alternative explanation may be provided by the data of Nielsen (1991) and Hildebrandt et al. (1994). Nielsen (1991) demonstrated that venous congestion in an elevated limb might actually augment blood flow. He observed that total arterial inflow ceased in limb segments that were elevated high enough above heart level that local diastolic pressure was 0. The energy of the systolic pulse of blood was effectively absorbed by the elastic compliance of the arterioles and expelled back towards the heart, a "windkessel" effect. When a venous cuff at pressures between 10-30 mmHg was inflated proximal to the elevated limb segment, he observed that blood flow

through the limb was restored. Hildebrandt et al. (1994) also observed a similar flow enhancing effect of inflating a venous cuff around the upper arm during arm elevation. In their study, the arm elevation was not as severe and there was still considerable flow without venous cuffing. These studies suggest that in the elevated limb, vessel collapse can occur which effectively increases the resistance to inflow, but that maintenance of venous pressure and volume reduces this effect and allows blood flow to occur. In agreement with this, others have also shown that venous distension reduces resistance to flow (Phillips *et al.*, 1955; Read *et al.*, 1958). This may explain the maintenance of FBF upon arm elevation when venous congestion was maintained in our study.

In terms of the observation of maintained FBF despite decreased local arterial pressure, three possibilities exist. First, there may have been an immediate myogenic vasodilatory response to the reduction in arterial transmural pressure. However, this seems unlikely as an explanation for the maintenance of FBF in the first 0-4 s of arm elevation, because myogenic vasodilation with reductions in arterial pressure does not respond that rapidly (Johnson and Intaglianetta, 1976; Borgstrom *et al.*, 1981; Jones and Berne, 1965). Second, if venous pressure does act as the downstream pressure, then arm elevation likely did not alter the local perfusion pressure gradient very much, since acute hydrostatic effects in arterioles and venules may have been similar, even when total venous volume was not allowed to change. Third, Saupe et al. (1995) have presented evidence that an arteriolar compliant region determines the effective back pressure to arterial inflow. Such a compliant region functions as a capacitor for arterial inflow. With each systolic pulse of blood, the region fills and pressure within it increases to match arterial diastolic pressure, such that during diastole no arterial inflow occurs. During this diastolic period, the compliant

region discharges blood into the capillaries. They observed that when diastole was prolonged, the subsequent systolic pulse was elevated as would be expected if the volume, and therefore the pressure, in this compliant region was reduced to a greater degree. We also observed an increased systolic brachial artery velocity pulse during the first 4 seconds of arm elevation (Figure 3.6) whether the venous congestion cuff was inflated or not. This is consistent with arm elevation reducing the pressure in this compliant region, i.e. the effective back pressure to arterial inflow. A reduction in the effective back pressure of this compliant region due to venous volume reduction in the venules may have allowed for a discharge of volume from the compliant region with a subsequent reduction in pressure. In this sense, venous pressure would have contributed to the effective downstream pressure for arterial inflow.

Evidence for a Withdrawal of Veno-Arteriolar Constriction with Arm Elevation

To date, the action of a veno-arteriolar reflex on arterial vascular tone has been examined in terms of the vasoconstriction induced when a limb is moved into the dependent position. This mechanism operates in both subcutaneous (Henriksen, 1991; Vissing *et al.*, 1997) and muscle (Henriksen and Sejrsen, 1977; Henriksen *et al.*, 1983) tissue. In addition, it appears that when muscle contractions maintain a reduced venous volume, the vasoconstriction on assuming limb dependency is abolished (Henriksen and Sejrsen, 1977). This suggests that removal of venous volume should result in a vasodilation. In our study we tested this by elevating the forearm from below to above heart level and either allowing or preventing venous drainage. We observed that, within 5-s of arm elevation a transient increase in FBF occurred which peaked by 8-s and then declined to levels that were still elevated above below heart FBF. Since mean arterial pressure did not change and since local arterial and venous pressure changes were for the most part complete

by this time, the increase in FBF could only be explained by a vasodilation. Two lines of evidence support this conclusion. First, in experiments where a venous congestion cuff was inflated to prevent changes in total venous volume, the increase in FBF was abolished. Second, we observed a 4.43-fold increase in flow when the arm was lowered during the time that the transient hyperemia above heart was observed. If no vasodilation was present, a 4.43-fold increase in the arterial-venous pressure gradient compared to baseline below heart would have been required. Given the arterial pressure of 118 mmHg in the below heart position and assuming that venule pressure was reduced to 0 with arm elevation, the venule pressure prior to arm elevation would therefore have to have been ~95 mmHg! Venous pressure measured at the elbow was ~20-25 mmHg, making such a value extremely unlikely given the low pressure gradients along the venous system (Rowell, 1993). Taken together, this evidence strongly suggests that venous emptying upon arm elevation initiates a transient vasodilation, and supports the hypothesis that such a vasodilation is mediated by a removal of vasoconstriction due to the veno-arteriolar reflex.

The transient nature of the FBF response to arm elevation has not been examined. It has been demonstrated that blood flow does not decrease with limb elevation above heart level up to a 30 mmHg drop in arterial perfusion pressure, indicating normal autoregulation up to this level (Nielsen, 1983). However, the flow response has not been measured on a beat by beat basis as we did with Doppler ultrasound. The evidence presented here suggests that there is a transient overshoot in the vasodilation initiated by the withdrawal of the veno-arteriolar vasoconstriction, which occurs over 9-10 s. After this, FBF remains slightly but significantly elevated above heart level compared to below for the remainder of a 2 min elevation period. This is consistent with the observations of hand skin blood flow by Petersen and Sindrup (1990).

Does Venous Pressure Contribute to Effective Downstream Pressure?

In a previous study, we simulated the mechanical venous emptying of muscle contraction via rapid 1-s inflation of an arm cuff and observed that FBF was immediately elevated upon release of the cuff when the arm was in the dependent position (Tschakovsky *et al.*, 1996). Others have also observed a flow enhancing effect of muscle contraction in the dependent position (Folkow *et al.*, 1971; Sheriff *et al.*, 1993). This effect has been attributed to the reduction in venous pressure accompanying venous emptying such that the arterial-venous pressure gradient is increased, thereby increasing arterial inflow (Laughlin, 1987). In this study, rather than mechanically emptying the forearm, we elevated it above heart level to drain blood from the forearm veins towards the heart before lowering it to restore arterial pressure under conditions of reduced venous pressure.

Figure 3.2 and 3.6 illustrate that the hyperemia upon arm lowering after 4 s of arm elevation was far greater than when venous volume had not been allowed to decrease. When venous drainage was allowed, the hyperemia on lowering had significant diastolic flow. This was a consistent observation across all subjects. However, when venous congestion had been maintained, the only observable difference in the velocity waveform compared with pre arm elevation was the slightly higher systolic pulse and the absence of a retrograde flow pulse following systole. These data clearly indicate that a reduction in venous volume was the predominant contributor to the immediate hyperemic response on arm lowering. The question was, how much of the effect could be attributed to the elevation in arterial-venous pressure gradient.

Peak VC elicited by arm elevation could be estimated as peak above heart FBF ÷ local arterial

driving pressure, assuming that venule pressure was ~0 mmHg (3.97 ml/100ml/min ÷ 86 mmHg = 0.046 ml/100ml/min/mmHg). In the below heart position, the mean arterial pressure estimated at mid-forearm level was ~118 mmHg. Unfortunately, measurements of venule pressure in the in vivo condition were not possible, therefore the gain in pressure gradient when the veins were emptied by transient arm elevation could not be determined. If we assume that venule pressure was ~0 in the arm above heart position and changed as much as arterial pressure on lowering, then it would be ~32 mmHg in the below heart position once the veins had filled. A VC of 0.046 ml/100ml/min with a below heart arterial-venous pressure gradient of 118-32 mmHg would have resulted in a FBF of 3.97 ml/100ml/min. The contribution of the increase in arterial-venous pressure gradient due to venous drainage could then be added to this by calculating flow as (118 mmHg - 0 mmHg) * 0.046 ml/100ml/min/mmHg. The results is a FBF of 5.4 ml/100ml/min, yet we observed 10.2 ml/100ml/min. There are two possible explanations for this. First, the calculated peak VC in the arm above heart position might be an underestimation of the actual VC immediately upon lowering. It is possible that with arm elevation, collapse of vessels occurred. This may have added to the resistance to flow since, for a given dilation, flow would only have occurred through open vessels. When the arm was lowered, the collapsed vessels might then have opened and VC would have increased. Second, venule pressure in the below heart position may have been substantially more than 32 mmHg. For this to have been the case though, arm elevation would have to have altered venous pressure more than arterial pressure. While the hydrostatic effect should be the same for both below heart, it may be that since venules are on the drainage side of the circulation, that once above heart level venous emptying added to the hydrostatic effect on venule pressure.

When the arm was lowered after 2 min compared to 4 s, peak FBF was 2.18 times baseline below heart when total forearm volume was maintained and 2.49 times when the veins had been allowed to drain. Cuff inflation prevented a change in venous volume when the arm was elevated. Therefore an increase in the arterial-venous pressure gradient when the arm was lowered could not have contributed to this hyperemia. During the 2 min of arm elevation, blood flow increased significantly, indicating that a vasodilation had occurred in response to arm elevation. In addition there is evidence that maintained venous congestion over a few minutes with a cuff inflated to 20 mmHg in the arm above heart position results in an immediate vasodilatory response upon cuff deflation (Walker *et al.*, 1967), and that this vasodilation results in a 2-fold increase in FBF. These investigators did not observe such an effect when venous congestion was maintained for 10 s. Therefore, the increased FBF observed on lowering the arm and releasing the cuff after 2-min of elevation and inflation might have been a consequence of vasodilation due to cuff release (Walker *et al.*, 1967).

Part of the explanation for the reduced hyperemia when venous drainage was allowed compared to lowering after 4 s could be that no contribution of the transient vasodilation due to removal of the veno-arteriolar vasoconstriction could occur at this time. However, since it appears that calculated VC in the above heart position is likely an underestimation when applied to the below heart condition under the conditions of this study, it was not possible to compare VC and arterial-venous pressure gradient contributions to the hyperemia upon arm lowering after 2 min vs. 4 s. The transient increase in FBF upon lowering the arm indicates that elevating the local pressure gradient for a given VC can increase FBF.

To summarize, the lack of reduction in FBF immediately upon arm elevation suggests that the

decrease in local arterial pressure was compensated for by similar decreases in venule pressure. Since not all of the hyperemia following acute arm elevation could be explained by increases in VC it appears that a decrease in venous pressure contributes to FBF elevation upon arm lowering.

Acute and Prolonged Forearm Elevation: Effect During Exercise

While there had been no reduction in arterial inflow immediately upon arm elevation in the resting forearm, there was a marked reduction in blood flow upon elevation during exercise (Figure 3.5, 3.7). This suggests that forearm arterial inflow was sensitive to reductions in arterial pressure during exercise when the vasculature was in a vasodilated state and there was considerable diastolic flow, but not at rest when the vessels were vasoconstricted to the point where there was no arterial inflow during diastole. Exercise was used in this study in an attempt to reduce venous volume and pressure in the arm below position (Stegall, 1966; Stick *et al.*, 1992; Pollack and Wood, 1949) so that on elevation of the arm, changes in hydrostatic pressure would occur predominantly on the arterial side. This would be in contrast to the resting condition where considerable reduction in venous volume and pressure would occur with arm elevation. The reduction in arterial inflow on arm elevation in the exercising but not the resting forearm might then be attributed to the greater change in arterial-venous pressure gradient that would have occurred.

By the second relaxation phase in arm elevation, there was a partial recovery of blood flow, but no further recovery if the arm remained elevated. This indicated that a vasodilation had taken place. This may have been due to an effect of reduced vasodilator metabolite washout, but this explanation seems unlikely considering that the response was rapid but incomplete. A second explanation might be that of a myogenic response of the arterioles to the alteration in transmural

pressure (Johnson and Intaglianetta, 1976; Shepherd, 1983). When the arm was returned to the below heart position, blood flow increased transiently above levels observed prior to arm elevation. This elevation in flow was rapidly returned to arm-down baseline, even following prolonged arm elevation where there had been a relative flow deficit compared to exercise with the arm below heart level. This suggests that changes in concentrations of vasodilator metabolites were likely not the cause and that perhaps a myogenic mechanism was again responsible for the adjustment in flow.

Venous Pressure-Volume Relationship

In order to assess the relationship between venous volume and pressure in the forearm as the veins were emptied and then refilled by altering forearm position relative to heart level, we used strain gauge plethysmography at the largest circumference of the forearm just distal to the elbow to determine % change in forearm volume and venous catheterization of an antecubital vein at the elbow. Changes in venous volume were inferred from changes in forearm volume and this approach has been used extensively by other investigators (Barendsen and van den Berg, 1984; Tripathi *et al.*, 1989; Bevegard and Shepherd, 1966). Both interstitial and intravascular volume changes can contribute to the observed % changes in arm volume measured by strain gauge plethysmography. Reductions in interstitial fluid in the forearm would rely on reabsorption into the vasculature at the capillary level where capillary filtration pressure is reduced upon arm elevation. Hildebrandt et al. (1993) have demonstrated that this process occurs at a rate of ~0.04 ml/100ml/min, and therefore its contribution can be ignored in our measurements and changes in forearm volume taken to represent predominantly changes in venous volume.

When the forearm was lowered following acute arm elevation, there was a rapid early increase

in volume, coincident with the transient hyperemia, followed by a slower steady increase over a period of time where inflow was at steady resting values. The time course of this refilling of the venous capacitance agrees with previous observations by (Barendsen and van den Berg, 1984) in the calf lowered to the dependent position following ~25 s of elevation above heart level. When the arm was lowered following 2 min of arm elevation, the time course of venous refilling was markedly extended. There was virtually no initial rapid increase in forearm volume since the hyperemia upon arm lowering was of a much smaller magnitude and duration than after 4 s of arm elevation. Thereafter there was a slow steady increase during the period of steady resting arterial inflow.

Venous pressure on the other hand showed a rapid return to below heart levels that appeared to be coincident with the duration of the transient hyperemia upon arm lowering (Figure 3.2,3.3,3.4). In Figure 3.4, when venous pressure and forearm volume are expressed as % change, it can be clearly seen that on arm elevation and venous emptying, volume and pressure change proportionally. However, on arm lowering, venous pressure increases more rapidly than venous volume. This effect was magnified if arm elevation was maintained for 2 min. In other words the veins exhibited a hysteresis in the pressure volume relationship with emptying and refilling. This effect is illustrated clearly in Figure 3.9, and agrees with data from Journo et al. (1992), who observed hysteresis in the forearm during slow increases and decreases in distention. Such a response indicates a viscous component in the venous wall which responds slowly to the changes in venous volume incurred by altering arm position relative to heart level.

Summary

The application of Doppler ultrasound in this study has allowed us to obtain beat by beat

measures of blood flow in response to alterations in limb position relative to heart level. We have demonstrated that elevation of a resting limb above heart level does not immediately affect FBF. However, a transient increase in blood flow is initiated within 5 s of elevation and it is mediated by a vasodilation that appears to be dependent on the emptying of venous volume. This supports the hypothesis of a veno-arteriolar reflex, but extends the concept to include a transient vasodilatory response upon withdrawal of this vasoconstriction. We have also demonstrated a transient hyperemia upon lowering of the arm that could not be entirely accounted for by changes in forearm vascular conductance that may have occurred with arm elevation. Therefore it is concluded that an increase in the local arterial-venous pressure gradient due to reductions in venous volume and pressure achieved during arm elevation also contributed to the hyperemia on lowering.

CHAPTER IV

-60 mmHg LBNP elicited increases in SNS activity: is the adaptation of blood flow to the exercising forearm compromised?

ABSTRACT

High levels of lower body negative pressure (LBNP) are normally associated with constriction of arterioles supplying forearm muscles. We tested the hypothesis that the greater vasoconstriction during LBNP would impair the adaptation of forearm blood flow (FBF) at the onset of exercise. 9 subjects lay supine with the lower part of the body sealed in the LBNP box. Their right arm was extended and supported ~15 cm below heart level. 5 min of forearm exercise (lifting and lowering 8 kg through 3.5 cm in a 1-s/2-s work/rest schedule) was performed during -60 mmHg LBNP (LBNP) and without LBNP (Control). LBNP was initiated 4-5 min prior to the start of data collection to achieve a stable baseline. Beat by beat forearm blood flow (FBF, determined by Doppler ultrasound, mean arterial pressure (MAP), and heart rate (HR) were collected. LBNP elevated resting HR by ~45%. MAP was not significantly changed, but diastolic pressure was elevated by ~10% and pulse pressure was reduced by ~20%. At rest, FBF (ml/min ± SE) was not different in LBNP vs. Control (33.5 ± 5.1 vs. 34.1 ± 5.7). However, the initial rapid increase in FBF which plateaued between 10-20 s was reduced in LBNP (99.6 ± 10.0 vs. 121.4 ± 12.8). This difference was quickly abolished during the second, slower FBF adaptation phase. During the last minute of exercise, FBF was again significantly reduced in LBNP (193.5 ± 13.7 vs. 204.6 ± 14.5). The data suggest that high levels of LBNP can compromise the initial rapid increase in blood flow. However, it appears that factors responsible for increasing forearm vascular conductance during the second, slower FBF adaptation phase to steady state are able to compensate and temporarily restore the normal FBF adaptation.

Key words: Doppler ultrasound, lower body negative pressure, exercise, sympathetic nervous system

INTRODUCTION

Increases in sympathetic outflow are normally associated with constriction of arterioles in resting skeletal muscles (Tripathi *et al.*, 1989; Tripathi and Nadel, 1986; Joyner *et al.*, 1990; Joyner *et al.*, 1990). However with the onset of exercise, accumulation of metabolic vasodilators is thought by some investigators to actively inhibit adrenergic effects on vascular smooth muscle (Hansen *et al.*, 1996; Remensnyder *et al.*, 1962). There is some evidence that this effect may be temporary. For example, Joyner et al. (1990) increased forearm sympathetic nerve activity (SNA) via upright standing compared to the supine posture and found that exercising forearm blood flow was not affected by elevated sympathetic tone during the first 5 min of exercise but was significantly decreased thereafter. Peterson et al. (1988) observed that the blood flow response during the first 2 min of exercise in sympathectomized rats was not altered compared to control, but that thereafter, control rats had a lower exercising blood flow. In contrast, Strandell and Shepherd (1967) observed a reduction in exercising forearm blood flow (FBF) during -60 mmHg lower body negative pressure (LBNP) throughout a 5 min exercise bout, although the magnitude of this reduction was less at higher exercise intensities. Shoemaker et al. (1997) investigated the FBF and muscle metabolic response to progressive 1 min step increases in rhythmic handgrip exercise in -60 mmHg LBNP and found that forearm blood flow was reduced at all work rates.

An important limitation in all of the studies in humans that examined the adaptation of FBF to exercise during elevated SNA has been the poor time resolution of strain gauge plethysmography (Joyner *et al.*, 1990; Strandell and Shepherd, 1967). Because strain gauge plethysmography requires that blood flow be measured during brief, intermittent pauses in exercise, it is therefore unable to provide clear information on the dynamic changes in blood flow during a rest to exercise

transition. In addition, it is not known how intermittent pauses in exercise might interact with elevated sympathetic tone. This limits the interpretation of FBF measured with strain gauge plethysmography during the adaptation from rest to exercise.

Doppler ultrasound overcomes the limitations of strain gauge plethysmography as it provides continuous beat by beat blood flow measurements during rhythmic exercise (Radegran and Saltin, 1998; Robergs *et al.*, 1997; Shoemaker *et al.*, 1997; Eriksen *et al.*, 1990; Walloe and Wesche, 1987). Therefore the purpose of this study was to apply Doppler ultrasound to investigate the effect of elevated sympathetic tone on the dynamic adaptation of blood flow at the onset of rhythmic, dynamic forearm exercise. We used -60 mmHg LBNP to elicit increases in forearm sympathetic tone. Studies conducted with this level of LBNP have demonstrated reductions in exercising FBF (Shoemaker *et al.*, 1997; Strandell and Shepherd, 1967), therefore we hypothesized that the sympathetic forearm vasoconstriction established at rest in -60 mmHg LBNP would impair the dynamic response of blood flow at the onset of rhythmic dynamic forearm exercise in humans.

METHODS

Subjects

Nine healthy subjects (8 males and 1 female, 24.7 ± 0.7 yrs, mean ● SE) participated in this study and gave written consent on a form approved by the Office of Human Research of the University after receiving full written and verbal details of the experimental protocol and any potential risks involved. Each subject came to the laboratory on one occasion prior to the experimental sessions in order to assess their tolerance for -60 mmHg LBNP and to familiarize them with the experimental protocol.

Experimental Design

Subjects arrived at the laboratory in a rested state at least 2 hours after eating. They assumed a supine position, with their right arm extended to the side approximately ~15 cm below heart level, and were sealed from the level of the supra-iliac crests down in a lower body negative pressure (LBNP) box (Figure 4.1). Since we were interested in muscle blood flow changes specifically, we reduced skin blood flow in the subjects by cooling their arm over a period of 30 to 40 minutes with the aid of a fan and in some cases a hand-held bottle of ice. When forearm blood flow velocity (pulsed Doppler) monitored during this period was observed to stabilize at minimal levels characterized by systolic pulse flow only and little variability, the ice bottle was removed. Experiments then began with forearm cooling maintained by the fan. Room temperature was between 21 and 23 °C during testing.

Exercise with the forearm consisted of smoothly raising and lowering an 8 kg weight through a vertical distance of 3.5 cm over a 1-s period in time with a signal light which set a work/rest duty cycle of 1 s/2 s. Within each work period, approximately 0.5 s was required for

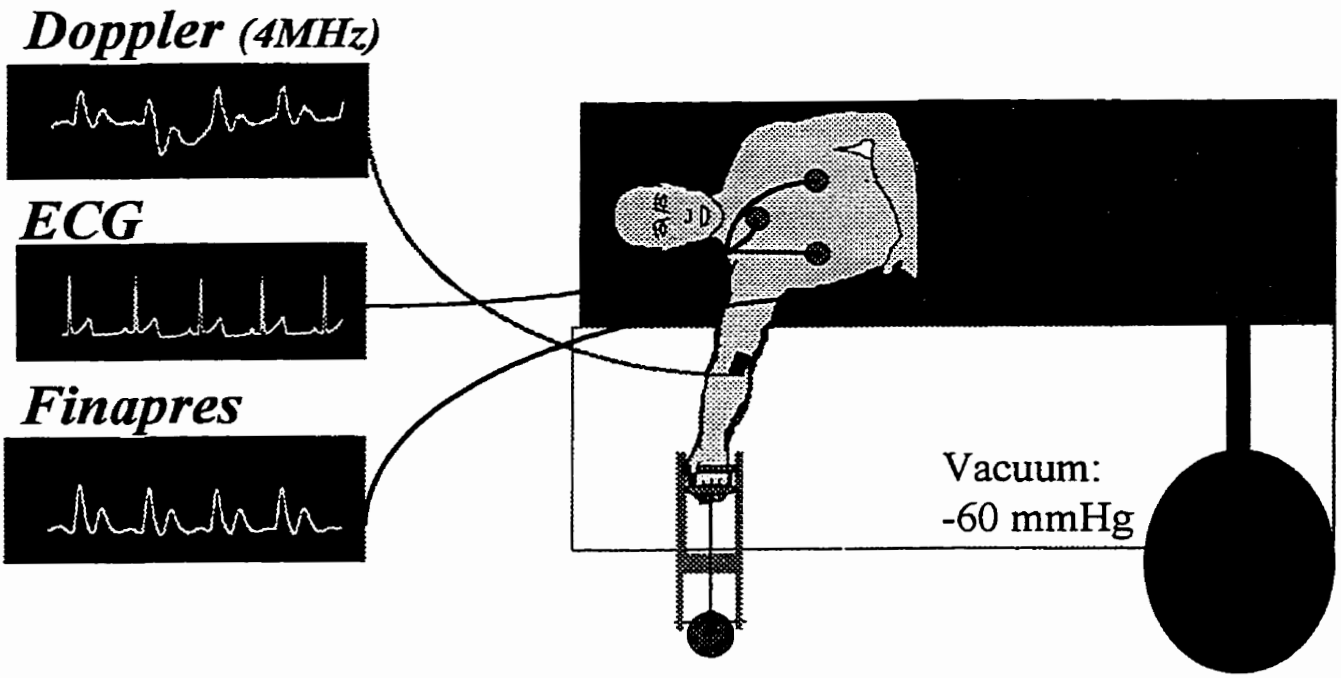

Figure 4.1 *An illustration of the experimental setup, viewed from above. The exercising forearm was ~15 cm below heart level. Exercise consisted of lifting and lowering an 8 kg weight a distance of 3.5 cm with a work/rest schedule of 1-s/2-s.*

each of lifting and lowering the weight. This exercise was performed with -60 mmHg LBNP and without LBNP (control). The order of the experimental conditions was counter-balanced among subjects. In each experimental condition subjects performed at least 2 trials. Doppler measures of brachial artery mean blood velocity were observed to be sure that a stable baseline was present and then data were collected for 1 min at rest followed by 5 min of forearm exercise. At least 10 min of rest occurred between each exercise bout during which mean blood velocity had recovered to previous resting values. During the LBNP condition, -60 mmHg LBNP began 4-5 min prior to the start of exercise and was terminated immediately at the end of exercise. Pilot work had indicated that it was difficult for subjects to withstand more than 9 min of LBNP without exhibiting signs of presyncope. To eliminate the effects of anticipation, subjects remained unaware of the time in any trial and were simply told at the appropriate time to begin or to cease exercise.

Data Acquisition

Heart rate (HR) and mean arterial pressure (MAP) were measured beat by beat. MAP was measured at heart level using a photoplethysmograph finger blood pressure cuff (Ohmeda 2300, Finapres, Lakewood, CO) on the middle finger of the left hand.

Forearm blood flow (FBF) was obtained beat by beat as the product of brachial artery mean blood velocity (MBV) and arterial cross sectional area:

$$\text{FBF (ml/min)} = \text{MBV (cm/s)} \bullet 60 \text{ s/min} \bullet \pi(\text{brachial artery diameter (cm)}/2)^2$$

Brachial artery blood velocity was measured with a 4-MHz pulsed Doppler ultrasound probe (Multigon Industries, model 500V, Mt. Vernon, NY) which was fixed to the skin over the brachial artery at the level of the antecubital fossa of the right elbow (Tschakovsky *et al.*, 1995).

With this placement and arm position, probe insonation angle relative to the skin was 45° and the brachial artery was approximately parallel with the skin. Arterial cross-sectional area was measured by a separate, linear 7.5 MHz echo Doppler ultrasound probe operating in B mode (Toshiba model SSH-140A, Tochigi-Ken, Japan) simultaneously with pulsed Doppler measures of MBV during the second or third trial in each condition. This probe was positioned ~9 cm proximal to the medial epicondyle, which was necessary to avoid acoustic interference between the probes. It has been shown previously in our laboratory that brachial artery diameters are not different between the two measurement sites (Shoemaker *et al.*, 1996). Imaged data were saved on video tape (Panasonic model AG-7300) for subsequent analysis. Arterial diameter was determined 4 times at rest and at 5 s, 10 s, 20 s, 30 s and thereafter every 30 s during forearm exercise. Diameter measurements at these times consisted of the average of 3 separate caliper measures of a frozen screen image of the brachial artery during diastole. All measurements were performed by the same operator.

Blood sampling. In 5 subjects a venous catheter (21 gauge, Angiocath) was inserted retrograde to flow into an antecubital vein draining the muscles of the forearm. Doppler imaging confirmed that this vein received blood from deep within the forearm. A three-way stopcock was fixed to the catheter. During the first trial of each experimental condition, 1 ml heparinized syringes were used to draw three 1 ml samples at rest, and two during the last 30 s of exercise. The samples were immediately gently agitated and stored in an ice bath. Within 1 hour of withdrawal, all blood samples were analyzed for PO_2 and hematocrit by selective electrodes in a blood gas-electrolyte analyzer (Nova StatProfile 9 Plus, Nova Biomedical Canada, Mississauga, ON). The analyzer was calibrated at regular intervals during the analysis period. [Hb] was

calculated, assuming it to be 33% of the mean corpuscular volume. Venous O_2 saturation and content were obtained from the output of the analysis system after application of standard equations. Arterial O_2 content was calculated based on the assumption that saturation remained constant at 97% and that hemoglobin was the same as in venous blood. Given the minimal demand placed on the cardiovascular system by moderate forearm exercise, it is reasonable to assume that arterial O_2 saturation would remain constant at a value observed on many occasions in our laboratory by ear oximetry. Forearm $\dot{V}O_2$ was determined from the Fick equation as the product of FBF and arteriovenous O_2 content difference, (a-vDO_2).

Data Analysis

For each subject, the diameter data were fit with an exponential regression to reduce random measurement error and provide continuous diameter estimates to match with the beat by beat MBV to allow calculation of FBF. HR, MBV and MAP data were saved continuously at 100 Hz on a dedicated computer via analog-to-digital conversion. For analysis, the beat by beat data were averaged into 3 s bins corresponding to the contraction/relaxation duty cycle and then averaged across all subject trials to determine the mean response profile. Values for HR, MAP and FBF reported at rest are the average of the 60 s rest period. Mean values at different times during forearm exercise are the average of 4 contraction/relaxation duty cycles for each subject (12 s average) except at 10 s and 20 s where they are the average of 1 contraction/relaxation duty cycle.

Statistical Analysis

The effects of LBNP on the hemodynamic variables over the course of the experimental trials was assessed with two way repeated measures ANOVA. Comparisons between control and

LBNP at specific times and within a condition compared to rest were performed with one way repeated measures ANOVA to test specific hypotheses (effect of LBNP at rest, effect of LBNP on the magnitude of the initial rapid increase in blood flow, effect of LBNP on the steady state exercise response, effect of duration of exercise in control or LBNP). The level of significance for ANOVA was set at $P<0.05$. All data are presented as means ±SE. All tests were completed with a commercial statistical package (SigmaStat 1.0, Jandel Scientific Corp.).

RESULTS

Systemic Cardiovascular Responses

Two way repeated measures ANOVA indicated a statistically significant interaction between main effects of time and condition for HR, MAP, systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP). -60 mmHg LBNP elevated resting heart rate compared to control by ~45% (86.1 ± 3.7 vs. 59.2 ± 4.2 beats/min, P<0.0001). This difference was maintained throughout the exercise period (Figure 4.2), as heart rate increased compared to rest in both control and LBNP. MAP was not different between control and LBNP at any time point and did not change over time in LBNP. However, MAP increased progressively in control and was significantly elevated compared to rest by 1 min of exercise (Figure 4.2). SBP was not different between conditions at rest, but progressively increased with exercise in the control condition while not changing in LBNP such that from 2-5 min of exercise it was significantly elevated in control (Figure 4.3). Compared to control, SBP was not different at rest in LBNP (128.1 ± 3.8 vs. 131.5 ± 3.6) while DBP was elevated (84.6 ± 2.5 vs. 77.2 ± 2.0 mmHg, P=0.01) and PP was reduced (43.5 ± 3.1 vs. 54.2 ± 2.4 mmHg, P=0.007) at rest. As exercise progressed, DBP was no longer statistically different between control and LBNP by 1.5 min of exercise (Figure 4.3). This was due to the fact that DBP did not change in LBNP but increased progressively in control.

Forearm Cardiovascular Responses

Measurements of brachial artery diameter proximal to the elbow showed no difference between LBNP and control at rest (4.3 ± 0.1 vs, 4.3 ± 0.1) , and diameters did not change with exercise in either condition. Two way repeated measures ANOVA indicated main effects of

condition and time on FBF. Specific analysis of the effect at rest revealed that FBF was not different between conditions (control: 34.1 ● 5.5 vs. LBNP: 33.5 ± 5.1 ml/min, P=0.741). With the onset of exercise, FBF increased rapidly in a biphasic manner in both conditions (Figure 4.2). However the magnitude of the initial rapid increase in blood flow was reduced in LBNP (99.6 ± 10.0 vs. 121.4 ± 12.8, P=0.007). This limitation of FBF due to LBNP was quickly overcome during the second phase of blood flow adaptation and flow was not different until the last minute of exercise (Figure 4.2). Evaluation of the steady state FBF indicated that the average FBF during the last minute of exercise was lower in LBNP (193.5 ± 13.7 vs. 204.6 ± 14.5, P=0.04).

Effect of LBNP on Resting and Steady State Exercise Forearm Oxygen Uptake

To determine whether steady state oxidative metabolism was affected by LBNP, venous blood samples during rest and the last minute of exercise were collected in 5 of the 9 subjects performing the rest to exercise transition in control and LBNP, and an additional 6 subjects in whom alternating 5 min bouts of control and LBNP were superimposed during rest and steady state exercise. Table 4.1 reports the effect of LBNP on resting and steady state FBF, oxygen extraction and calculated forearm $\dot{V}O_2$ for these 11 subjects. Oxygen extraction was increased in LBNP both at rest and in steady state exercise. Calculated forearm $\dot{V}O_2$ was not different between control and LBNP at rest or during exercise, indicating changes in O_2 extraction compensated for any changes in FBF to maintain oxidative metabolism.

Instantaneous Blood Velocity Profile

The lack of difference in the blood flow at rest was unexpected. During data collection it was observed that there was often a greater amount of retrograde flow in LBNP at rest and that the systolic peak velocity appeared to be reduced. This prompted an examination of the

instantaneous beat velocity and blood pressure profiles. Figure 4.4 provides an example of the instantaneous blood velocity at rest in three subjects. The characteristics of the blood velocity pulse varied somewhat from subject to subject. However, the potential interaction of heart rate in determining resting FBF at a given arterial pressure was apparent. It can be seen that the peak systolic blood velocity was reduced in LBNP, and there was generally a greater back flow pulse. However, what also became apparent was the absence of blood flow during diastole regardless of condition. While the actual volume of blood entering the arm per beat appeared reduced, the increased heart rate decreased the diastolic time of zero flow in LBNP.

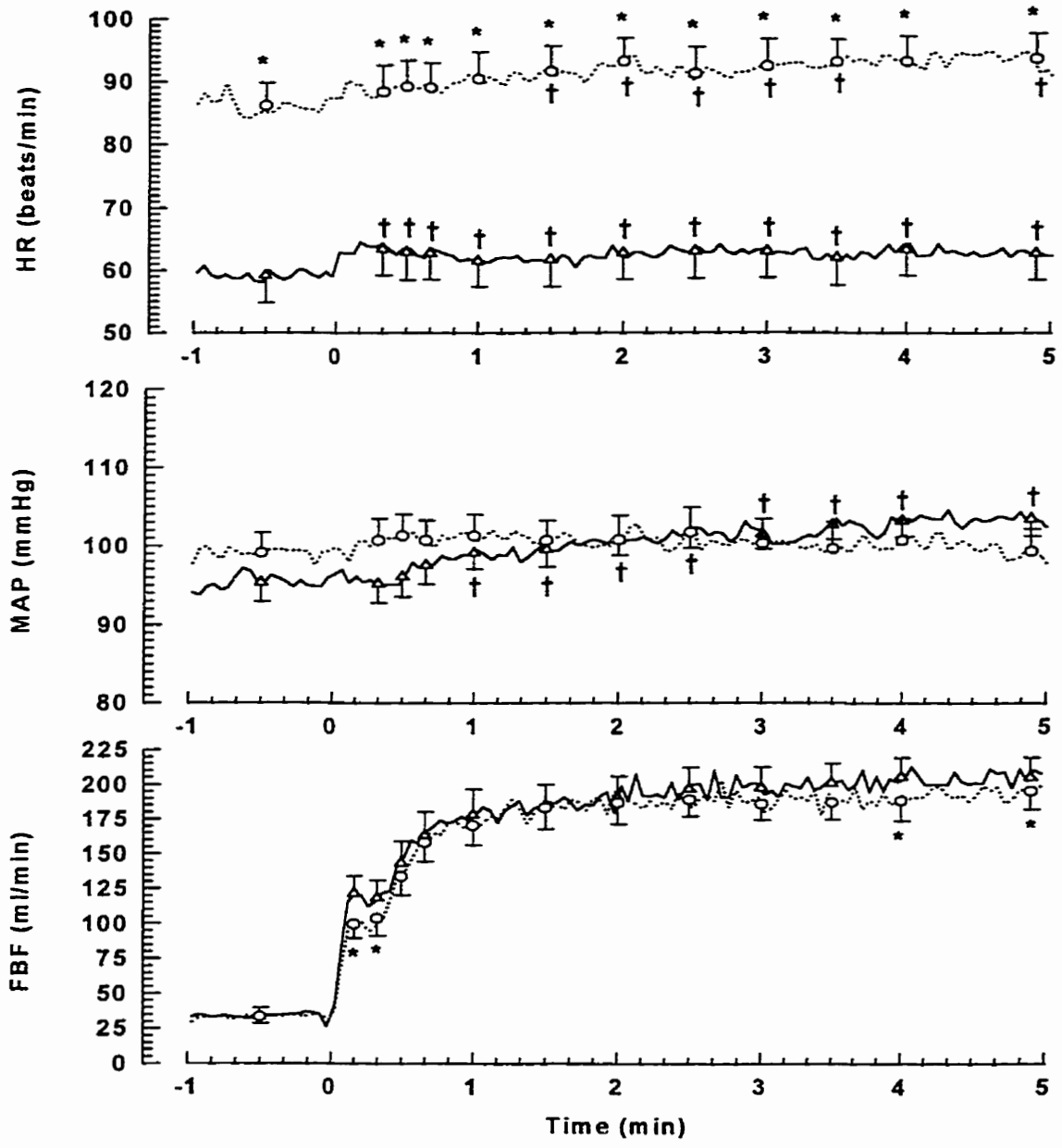

Figure 4.2 *Continuous average response (n=9) of heart rate (HR), mean arterial pressure at heart level (MAP) and brachial artery forearm blood flow (FBF). Control (——Δ——) and -60 mmHg LBNP (····o····). Exercise began at t= 0 min. *Significantly different from control. †Significantly different from rest within a condition, P<0.05.*

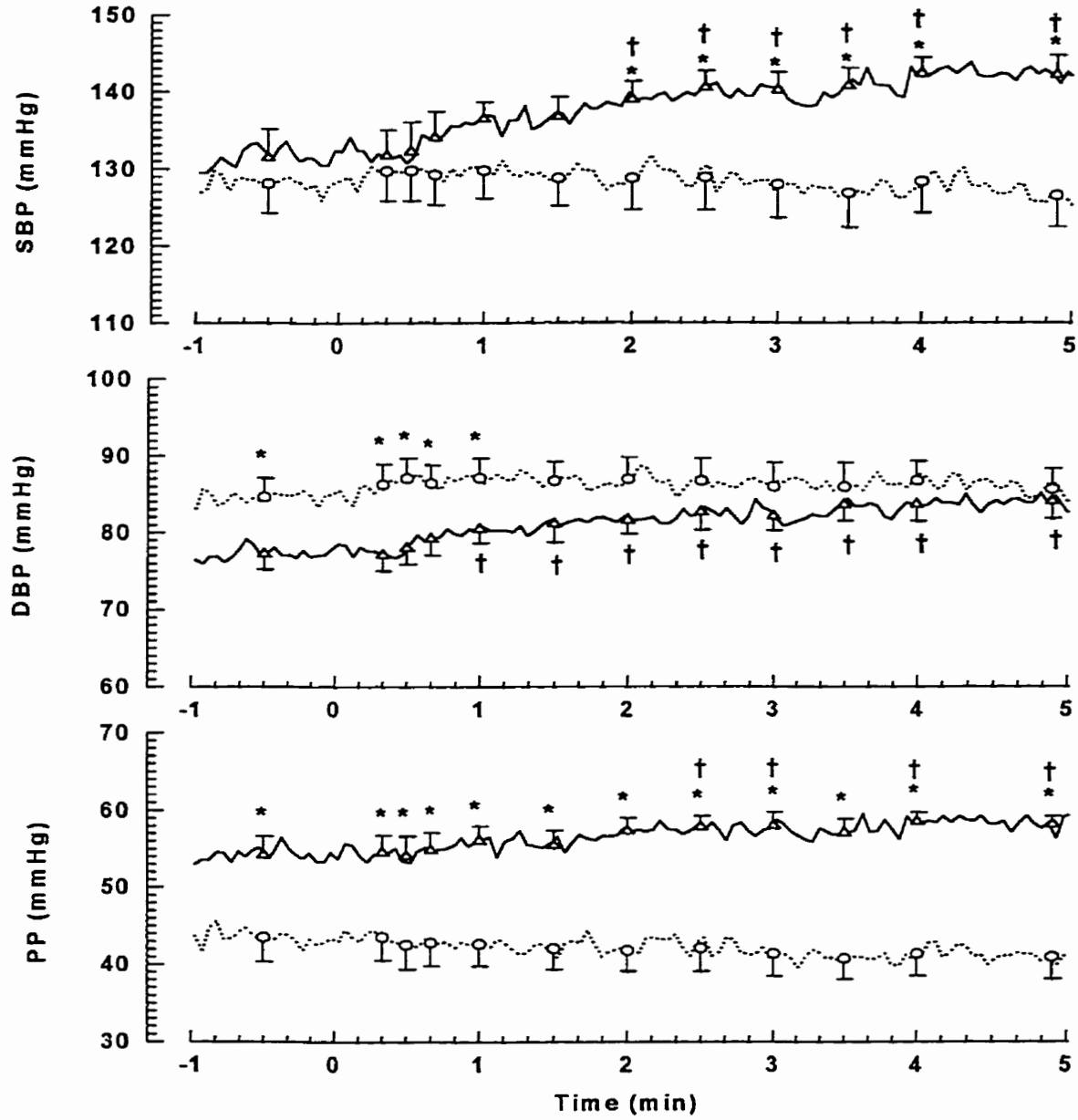

Figure 4.3 *Continuous average response (n=9) of systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP) at heart level in control and -60 mmHg LBNP. Control (——Δ——) and -60 mmHg LBNP (····o····). Exercise began at time = 0 min. *Significantly different from control. †Significantly different from rest within a condition, P<0.05.*

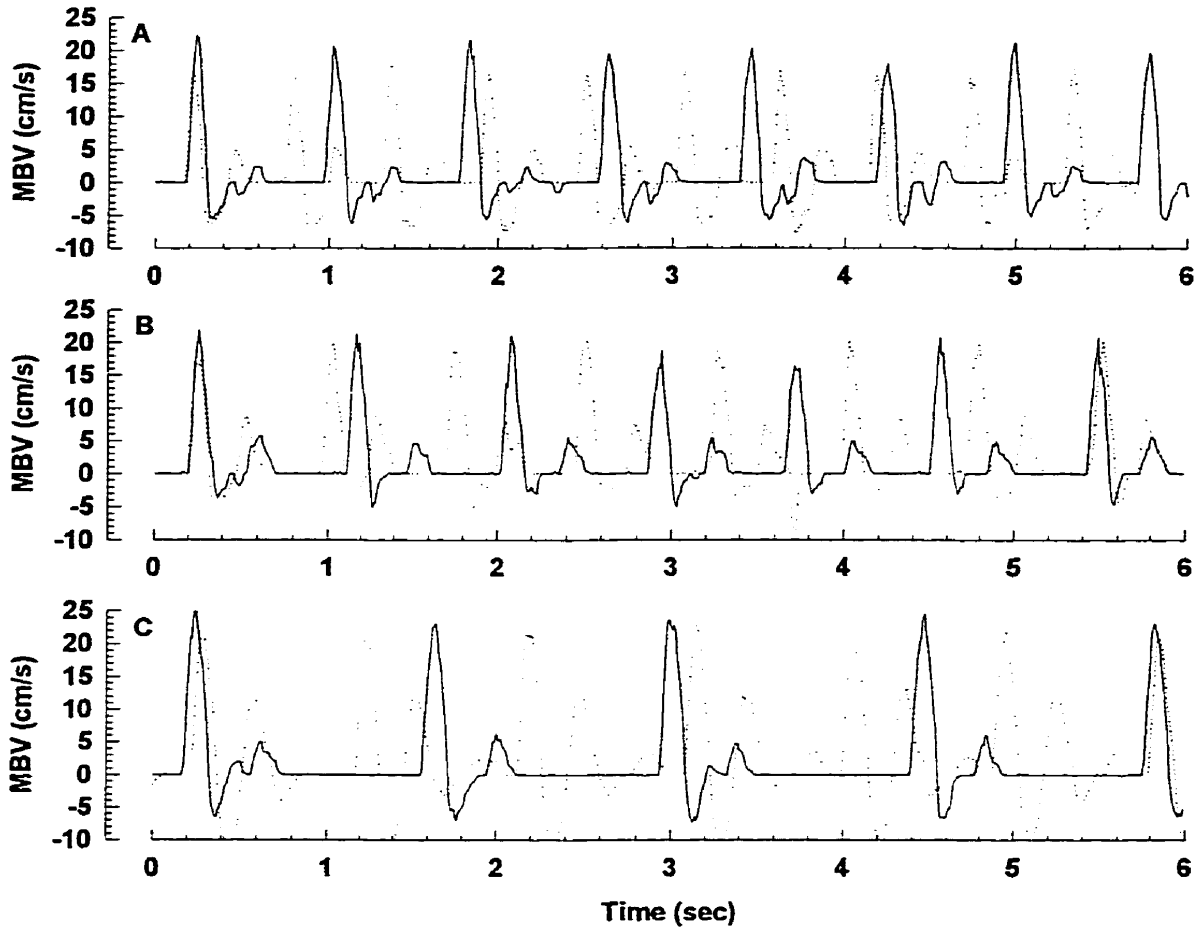

Figure 4.4 *Instantaneous mean blood velocity (MBV) profiles during rest for subject MS1 (A), subject MS2 (B) and subject BR (C). Control (——) and -60 mmHg LBNP (·····). A reduced systolic peak and greater retrograde and anterograde velocity fluctuations following the systolic pulse are evident in the LBNP condition, indicating a reduced forearm vascular conductance.*

Table 4.1 *Average values of forearm blood flow, arterial-venous oxygen difference and forearm $\dot{V}O_2$ at rest and 5 min of exercise.*

	Rest						Exercise Steady State					
Subject	FBF (ml/min)		a-vDO$_2$(ml/100ml)		$\dot{V}O_2$ (ml/min)		FBF (ml/min)		a-vDO$_2$ (ml/100ml)		$\dot{V}O_2$ (ml/min)	
	Control	LBNP	Control	LBNP	Control	LBNP	Control	LBNP	Control	LBNP	Control	LBNP
MS2	27	21	4.3	9.2	1.2	1.9	143	128	12.1	14.5	17.3	18.7
MA1	21	25	4.9	11.8	1.1	3.0	168	148	12.6	15.0	21.1	22.3
JC	18	25	9.3	9.2	1.7	2.4	165	170	12.0	14.6	19.8	24.9
AB	16	16	8.5	13.8	1.3	2.3	210	191	14.5	16.0	30.3	30.7
CC	19	21	8.1	10.2	1.6	2.2	209	213	9.4	12.4	19.7	26.5
AH	26	29	6.4	3.5	1.7	1.0	193	152	11.8	13.5	22.8	20.5
MC	23	16	5.6	7.4	1.3	1.2	133	120	12.0	13.1	16.1	15.8
JG	7	7	8.7	12.4	0.6	0.9	139	142	11.8	12.1	16.4	17.2
MA2	44	17	6.9	7	3.0	1.2	194	159	11.0	14.2	21.3	22.5
AF	25	19	8.2	13	2.1	2.6	234	208	14.0	16.1	32.7	33.3
KM	17	11	4.6	6.3	0.8	0.7	97	94	13.4	14.2	12.9	13.3
Mean	22	19	6.9	9.4*	1.5	1.8	171	157*	12.2	14.1*	20.9	22.3
±SE	±2	±2	±0.5	±1.0	±0.2	±0.2	±12	±11	±0.4	±0.4	±1.8	±1.9
P value	0.288		0.014		0.376		0.013		<0.0001		0.0925	

DISCUSSION

We have shown that an increase in sympathetic tone during -60 mmHg LBNP was associated with a reduction in the magnitude of the initial rapid increase in forearm blood flow at exercise onset. However, it appears that vasodilatory influences associated with the second adaptation phase of blood flow were able to compensate for the initial flow deficit such that blood flow adaptation in -60 mmHg LBNP rapidly matched that during control. The application of Doppler ultrasound in this study allowed us to examine the effect of -60 mmHg LBNP induced increases in sympathetic tone on the dynamic adaptation of forearm blood flow (FBF) during a rest to exercise transition. This method overcomes the limitations of strain gauge plethysmography in which brief, intermittent pauses in exercise are required to measure limb blood flow. The biphasic nature of the forearm blood flow response to exercise has been well characterized (Shoemaker *et al.*, 1998; Shoemaker *et al.*, 1996; Shoemaker *et al.*, 1996). The initial rapid increase which plateaus within 5-10 s is a result of both mechanical factors and vasodilation (Tschakovsky *et al.*, 1996; Radegran and Saltin, 1998). This is followed by a second, slower increase to steady state is initiated 15-20 s after the onset of exercise.

Central Cardiovascular Responses

As expected, -60 mmHg LBNP provided a substantial challenge to pressure regulating reflexes. HR was observed to increase by ~45% as part of the compensation for the reduction in stroke volume induced by LBNP (Abboud and Thames, 1983). The reduction in pulse pressure in LBNP likely reflected the combination of reduced stroke volume, increased total peripheral resistance and an increase in heart rate. The moderate increase in MAP with forearm exercise in the control condition was due to increases in both SBP and DBP. In LBNP, these increases with

exercise were not observed, suggesting that the mild pressor response elicited by this intensity of forearm exercise was hindered with LBNP.

Forearm Blood Flow Response: Rest

Blood flow through the forearm vascular bed is described by a form of Ohm's law for the circulation

$$\dot{Q}_a = \Delta P \cdot VC$$

such that arterial inflow ($\dot{Q}_a$) is determined by the arterial-venous pressure gradient (ΔP) and the vascular conductance of the resistance vessels. The FBF response in control vs. LBNP in this study will now be discussed with this model as a reference point.

Resting FBF was not altered by -60 mmHg LBNP in this study. This is in contrast to other studies in humans where sympathetic activity has been elevated by upright posture (Joyner *et al.*, 1990) mild LBNP (Tripathi *et al.*, 1989; Tripathi and Nadel, 1986) or -60 mmHg LBNP (Strandell and Shepherd, 1967). There are a number of differences between our study and these previous investigations that might explain this discrepancy. First, in these studies venous occlusion strain gauge plethysmography was used to measure blood flow, so the forearm was elevated above heart level to allow venous drainage. This means that the veins are virtually empty in both control and LBNP conditions during rest and therefore the ΔP would likely not be different. Therefore, differences in FBF at rest would be related exclusively to differences in VC. In our experiment, the arm was in the dependent position. Since LBNP has been shown to reduce forearm venous volume (Tripathi *et al.*, 1989), -60 mmHg LBNP could reduce venous pressure and effectively increase ΔP compared to control. This might offset the effect of reduced VC in LBNP on resting FBF. Another factor that might play a role in the measurement of FBF with

strain gauge plethysmography is that a venous occlusion cuff is inflated to prevent venous outflow and therefore arterial inflow is represented by increases in arm volume as the capacitance vessels fill. One possibility for the consistent observation of lower FBF in LBNP when measured with VOSGP (Tripathi *et al.*, 1989; Joyner *et al.*, 1990; Strandell and Shepherd, 1967) might be the reduced venous compliance that occurs with increased sympathetic activity (Rothe, 1983; Tripathi *et al.*, 1989). There is extensive evidence documenting sympathetic innervation of veins (for review see Rothe (1983)). Also, it has been demonstrated that venous volume at a venous pressure of 30 mmHg is reduced by over 20% at -50 mmHg LBNP (Tripathi *et al.*, 1989), indicating a reduced venous compliance in LBNP. We have shown previously that the cuff induced venous congestion artificially reduces arterial inflow by the second heart beat during exercise (Tschakovsky *et al.*, 1995), and this might be magnified in LBNP if venous compliance is reduced. To determine whether an effect of arm position or venous congestion might occur, we measured FBF at rest in an elevated arm by both strain gauge plethysmography and Doppler ultrasound in 5 subjects. Both methods indicated a significant reduction in FBF with -60 mmHg LBNP of 34% (Doppler LBNP 21.1 ± 3.7 vs. control 31.9 ± 5.7 ml/min, P=0.03; strain gauge LBNP 1.4 ± 0.2 vs. 2.1 ± 0.1 ml/100ml/min, P=0.046). This would appear to support an effect of arm position on the resting blood flow response in LBNP vs. control but not an effect due to venous cuff inflation.

Another condition unique to our study was the substantial effort to reduce skin blood flow by cooling the forearm over a period of 30-40 min. This resulted in low FBF in which there was consistently no diastolic flow. Other studies investigating the effect of LBNP on FBF typically exhibit resting control flow of 4-6 ml/100ml/min (Tripathi and Nadel, 1986; Strandell and

Shepherd, 1967; Joyner *et al.*, 1990), whereas strain gauge measures of blood flow in this study were ~1.5-2.5 ml/100ml/min. It has been demonstrated that skin blood flow contributes to the progressive decrease in FBF from -10 to -50 mmHg LBNP, but that muscle blood flow does not decrease any further beyond -20 mmHg (Tripathi and Nadel, 1986). Additionally, it has been observed that the reduction in blood flow in a warm forearm during LBNP is substantially greater than when the arm is cooled (Crossley *et al.*, 1966). This suggests a substantial role for skin vasoconstriction in the response to LBNP. However, others have provided neurophysiological evidence which indicates that skin sympathetic vasoconstrictor activity does not increase in LBNP (Vissing *et al.*, 1997; Vissing *et al.*, 1994). It may be that a reduction of skin blood flow with LBNP occurs for other reasons than a direct sympathetic vasoconstrictor response. One possibility as suggested by Vissing et al. (1997) is that LBNP mediated reductions in forearm skin blood flow are part of a thermoregulatory response brought on by the cooling effect of airflow around the lower body created by LBNP. Another possibility is that the collapse of the skin veins that would be expected to occur as LBNP shifts venous volume out of the arm (Tripathi *et al.*, 1989) would result in an increase in resistance to flow in the veins (Rothe, 1983). Therefore, to determine whether cooling of the forearm might explain our observations of no difference in resting blood flow between control and LBNP, the resting FBF response to the addition of -60 mmHg was measured when the forearm was warm and after it had been cooled.

Figure 4.5 summarizes the resting FBF response as measured by Doppler ultrasound in a transition from control to 8 minutes of -60 mmHg LBNP in 4 subjects. The results indicate a reduction in FBF (ml/min ± SE) with application of LBNP both when the forearm was warm (58 ± 13 to 30 ● 5, P=0.13) and when it had been cooled (31 ± 4 to 16 ± 3, P=0.02). While the

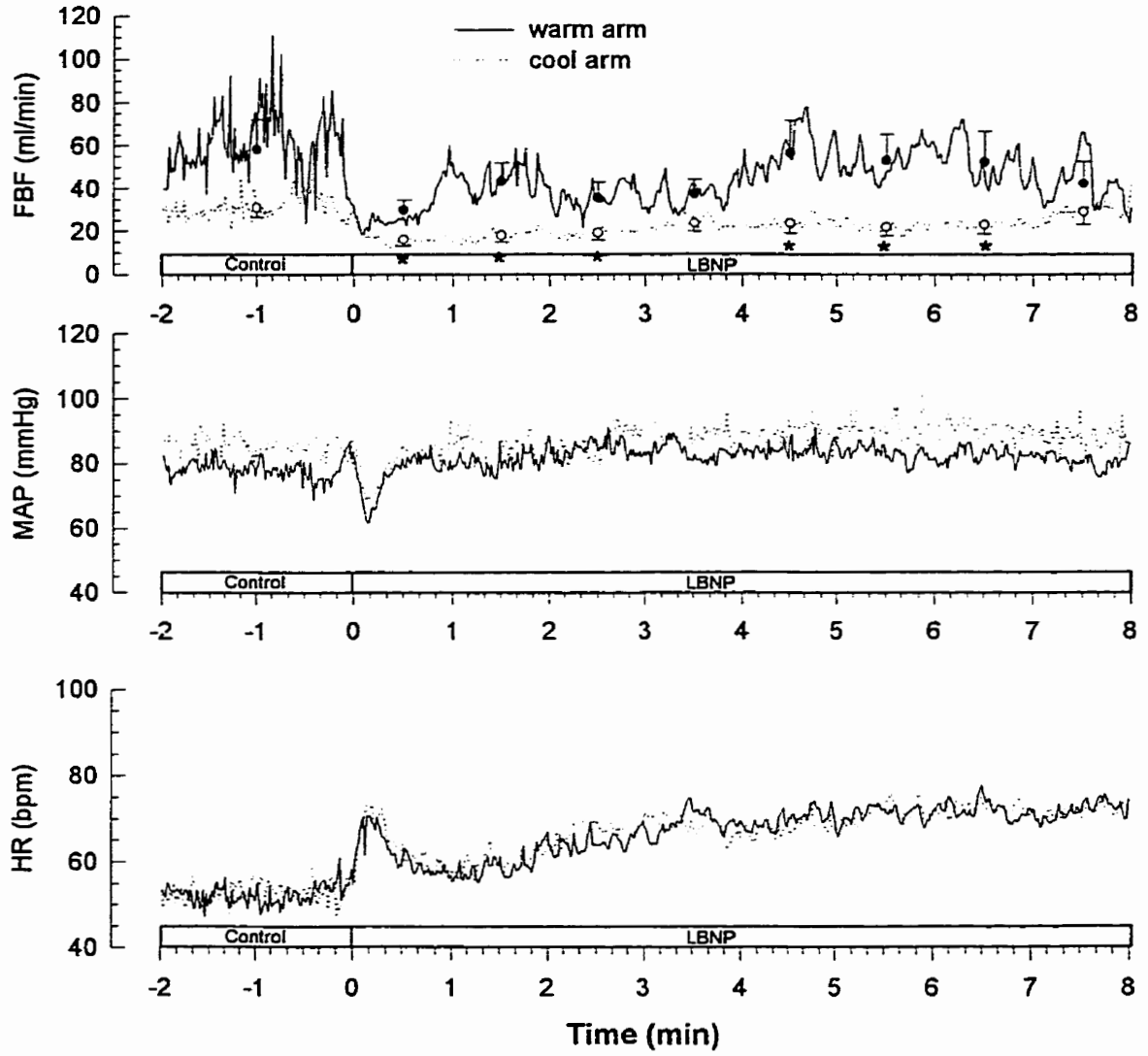

Figure 4.5 *Comparison of warm and cool arm response to the addition of -60 mmHg LBNP at rest. * Significantly different from rest within a condition (P<0.05) (n=4). Upper panel is forearm blood flow with symbols representing a 2 min avg in control and 1 min avg in LBNP. 2nd and 3rd panels are mean arterial pressure (MAP) and heart rate (HR) continuous responses.*

response in the warm arm is not statistically significant, this is likely due to the large variability and limited number of subjects. Power calculations indicate that, given the variability of the data, 10 subjects would be required to achieve statistical significance. In both conditions, the MAP and heart rate HR responses over time were identical, and FBF decreased with the initial reduction in MAP. As LBNP continued and MAP recovered, FBF showed a great deal of temporal variability in the warm arm condition, while there was little variability when the arm was cooled. By 8 min of LBNP, FBF in the cool arm was not different from FBF prior to the onset of LBNP. However blood flow in the warm arm, while being highly variable, also showed the ability to recover to near control levels. This evidence indicates that the absolute reduction in FBF with LBNP is less in a cooled arm, and suggests that arm cooling may have contributed to the resting FBF observations in our study. Perhaps more importantly , this evidence suggests an effect of time on the FBF response to LBNP that is consistent with the observations of a "sympathetic escape" found by Joyner et al. (1990). In their study, a partial recovery of FBF occurred over a 7 min period following the onset of -15 mmHg LBNP despite a maintenance of elevated sympathetic activity, indicating that the resistance vessels became less responsive to a given level of sympathetic activity.

Taken together, it would appear that the lack of difference in FBF observed at rest in our study compared with others might be explained by a combination of three factors. First, forearm exercise was performed in the dependent arm position. This meant that during LBNP a reduction in venous pressure could be achieved, improving the local ΔP and partially offsetting the decrease in vascular conductance that would be expected with elevated forearm sympathetic activity. Second, the arm was substantially cooled, minimizing the change in skin blood flow that would

contribute to the reduction in FBF with LBNP. Third, over the 4-5 min period of LBNP prior to the initiation of resting FBF measurements, a reduction in the responsiveness of the forearm resistance vessels may have occurred, i.e. a "sympathetic escape".

Effect of Elevated Heart Rate on Resting FBF

One final possible explanation for the lack of reduction in resting FBF in LBNP, where elevated forearm vasoconstriction would be expected, might be related to the elevated heart rate. This serves to reduce the time of diastole in which no inflow into the cooled forearm occurs at rest (Figure 4.4). Saupe et al. (1995) have provided evidence for a compliant region in the arteriolar circulation which receives blood during systole and continues to discharge blood into the capillaries during diastole. This region effectively stores the energy of arterial pressure in its elastic walls, and would be expected to release the blood at a rate proportional to the volume of blood it contained. The pressure in this compliant region would act as the effective downstream pressure for arterial inflow. At rest it is exceeded only during systole, explaining why flow into the forearm occurs only during systole. With LBNP, the amount of blood "injected" into this compliant region was reduced per beat, i.e. arterial inflow at the same mean arterial pressure was less per beat. This was likely due to a combination of a reduced stroke volume and an increased resistance to flow upstream of the compliant region. However with LBNP, diastolic time was also reduced considerably due to the ~25 beats/min increase in heart rate. It may be that the larger number of systolic pulses of blood in the LBNP condition, though each was reduced compared to control, compensated for a decrease in forearm vascular conductance. Given that such a compliant region results in zero inflow into the arm despite significant diastolic pressure, it may be that the mean arterial pressure does not adequately represent the effective pressure

gradient in a pulsatile pressure system where no flow is occurring during the diastolic phase and therefore forearm vascular conductance calculations based on it would not properly reflect the state of the vasculature.

FBF Response: Adaptation to Exercise

In agreement with previous studies of forearm (Shoemaker *et al.*, 1997; Shoemaker *et al.*, 1996; Hughson *et al.*, 1997) and leg exercise (MacDonald *et al.*, 1998; Shoemaker *et al.*, 1996; Shoemaker *et al.*, 1994) we observed a biphasic FBF response at the onset of exercise in both control and LBNP conditions. Such a biphasic response is characterized by an initial rapid increase in flow which plateaus within 10 s, followed by a further slower increase to steady state levels which begins ~15-20 s after exercise onset. This biphasic response suggests that at least some of the control mechanisms responsible for the immediate increase in flow are different from ones responsible for the continued increase in flow. We have recently demonstrated that the initial phase is determined by both the mechanical effect of the muscle pump, which increases the arterial-venous pressure gradient by emptying the veins, and as yet unidentified mediators of an early vasodilation (Tschakovsky *et al.*, 1996; Shoemaker *et al.*, 1998). Further increases during the second phase are thought to be mediated primarily by metabolic vasodilation (Delp and Laughlin, 1998).

In this study, the magnitude of the initial increase in FBF was significantly reduced in LBNP vs. control by ~25%. Local blood flow depends on VC and the local ΔP, therefore LBNP must have resulted in i) a reduced initial vasodilation or ii) a smaller gain in ΔP from rest to exercise or iii) a combination of the two. The early vasodilation may have been blunted by the elevated background of sympathetic tone in the forearm induced by LBNP (Joyner *et al.*, 1990; Baily *et*

al., 1990; Sundlof and Wallin, 1978). In support of this, Klabunde et al. (1986) using an in situ dog gracilis muscle preparation have demonstrated an attenuated hyperemia following a single muscle contraction under conditions of increased sympathetic stimulation. There is also evidence to suggest that LBNP might reduce the magnitude of the increase in ΔP from rest to exercise. In this study the forearm was in the dependent position. Therefore, a muscle pump contribution to the initial FBF adaptation phase would be anticipated (Tschakovsky *et al.*, 1996). During LBNP, the resting forearm volume is decreased (Tripathi *et al.*, 1989), indicating that venous volume is reduced. It is therefore probable that pressure is also reduced. If so, the increase in the local pressure gradient that can be achieved by muscle contraction at the onset of exercise would be reduced compared to control. This would therefore reduce the magnitude of the initial FBF increase at the onset of exercise in LBNP vs. control.

It is clear from Figure 4.2 that the reduction in FBF during the initial adaptation phase in LBNP is rapidly abolished during the second, slower adaptation phase. Since we would not expect ΔP during exercise to be different between control and LBNP, the similarity in FBF indicates that vascular conductance is probably also the same. This suggests that vasodilatory mechanisms responsible for this stage of blood flow adaptation were able to quickly compensate for the blunting effect of LBNP on the initial FBF response. Since Anrep and von Saalfeld (1935) first provided evidence that a vasodilator substance was released within a few seconds of the initiation of muscle contraction, many substances which can exert vasodilatory effects have been identified (see Shepherd (1983) for review). However it is still not clear which are essential for the adjustment of exercise hyperemia. Furthermore, those that may be involved in the early adjustments of vascular conductance are likely different from those responsible for later

adjustments (Laughlin *et al.*, 1996). Mechanisms which exert a sympatholytic effect have also been identified (for review see Shepherd and Vanhoutte (1981)). There is substantial evidence for a functional symptholysis in humans (Hansen *et al.*, 1996; Joyner *et al.*, 1990) and animals (Buckwalter and Clifford, 1998; Thomas *et al.*, 1994; Remensnyder *et al.*, 1962; Thomas *et al.*, 1994). Joyner (1990) has observed that intermittent measures of exercising forearm blood flow were not different in upright (elevated forearm sympathetic activity) vs. supine for the first five minutes of exercise. In addition, Hansen et al. (1996) have demonstrated a maintenance of tissue oxygenation in the exercising forearm under increased muscle sympathetic nervous activity induced by -20 mmHg LBNP unloading of the cardiopulmonary baroreceptors. However, there are also indications that this sympatholytic effect may not last for more than the first few minutes of exercise under some conditions (Peterson *et al.*, 1988; Joyner *et al.*, 1990). In this study, the data support the existence of a functional sympatholysis which opposes the increased forearm sympathetic activation after the first 20 s of exercise.

Deep Venous Oxygen Content

Venous catheterization with a 1.5 inch, 21 gauge catheter was performed in a retrograde direction to venous flow in an attempt to optimize sampling of blood from muscle tissue. In addition, the arm was cooled to minimize skin blood flow. In this study, no wrist occlusion was performed. Corcondilas et al. (1964) had suggested the necessity of such an intervention to avoid venous contamination by blood returning from the hand. However, examination of the resting venous oxygen saturation in the control condition of ~60% agrees with observations of Corcondilas et al. (1964) with the wrist cuff in place. This suggests that arm and hand cooling and catheter placement were likely successful measures for minimizing skin flow contamination at

the catheter site in this study.

Venous oxygen content was consistently lower in LBNP both at rest and in exercise, such that calculated a-vDO_2 was also lower in LBNP (Table 4.1). Since the forearm position and work rate was identical between control and LBNP, it would be expected that the oxygen consumption should not be different. In support of this the calculated forearm $\dot{V}O_2$ was not different between conditions at rest and steady state exercise, indicating that the measured oxygen extraction was appropriate for the FBF response in control vs. LBNP in this study. These results are consistent with Strandell and Shepherd (1967) who also observed a maintenance of forearm $\dot{V}O_2$ at rest and in exercise during -60 mmHg LBNP.

Conclusions

In summary, this study has provided continuous blood flow measures during the transition from rest to exercise under conditions of elevated sympathetic nervous activity due to -60 mmHg LBNP. In contrast with a number of other studies (Tripathi *et al.*, 1989; Hansen *et al.*, 1996; Tripathi and Nadel, 1986; Strandell and Shepherd, 1967), we did not observe a reduction in blood flow at rest with LBNP. However, our study conditions differed from previous investigations in humans. Therefore our observations might be explained by a combination of 1) the dependent position of the arm allowing LBNP to improve resting ΔP and compensate for a reduced forearm vascular conductance 2) the effect of arm cooling 3) a sympathetic escape during the 4-5 min period of LBNP prior to the onset of resting FBF measurements and 4) the large increase in heart rate observed in this study which reduced the diastolic time during which no arterial inflow occurred.

The initial rapid phase of blood flow adaptation may have been reduced in LBNP due to 1) a

reduced gain in the local ΔP with the onset of muscle contractions, 2) blunted vasodilation due to elevated forearm sympathetic vasoconstrictor activity, or a combination of both. The rapid recovery of blood flow in LBNP to match that in control during the second adaptation phase suggests that local vasodilatory factors are capable of compensating for the initial flow deficit induced by LBNP, perhaps through sympatholytic effects.

CHAPTER V

Ischemic muscle chemoreflex response elevates blood flow in non-ischemic exercising muscle

(Submitted to Am.J.Physiol., September, 1998)

ABSTRACT

We tested the hypothesis that forearm blood flow might be reduced during forearm exercise when sympathetic nervous activity (SNA) was elevated by calf exercise during calf circulatory occlusion (CE+O). Brachial artery forearm blood flow and mean arterial pressure were measured beat by beat during rest and forearm exercise. CE+O initiated prior to 5 min of forearm exercise (condition A) increased mean arterial pressure by 24% and reduced resting forearm vascular conductance by 24% such that forearm blood flow remained the same as in control (condition C). With the onset of forearm exercise, the difference in forearm vascular conductance between condition A and condition C was abolished, consequently the forearm blood flow adaptation to exercise was ~20-30% greater in condition A due to the elevated mean arterial pressure. Gradual stimulation of the chemoreflex by the addition of CE+O at 3 min of a 9 min bout of forearm exercise (condition B) did not affect forearm vascular conductance, such that progressive elevations in mean arterial pressure resulted in proportional increases in forearm blood flow. Chemoreflex-mediated increases in systemic SNA appear to affect resting forearm vascular conductance. However, evidence from this study suggests that local factors responsible for initiating and maintaining vasodilation during moderate, small muscle mass exercise can quickly override this vasoconstrictor influence such that exercising forearm blood flow is elevated due to elevations in mean arterial pressure.

Keywords: vasoconstriction, vasodilation, blood pressure, blood flow, exercise, sympathetic nervous system

INTRODUCTION

The muscle chemoreflex is a powerful mechanism for elevating systemic sympathetic nervous activity (SNA) (Rowell, 1997; Joyner, 1992; Rowell and O'Leary, 1990). Metabolic by-products of muscle contraction related primarily to anaerobic metabolism stimulate chemosensitive muscle afferent nerve fibres present in skeletal muscle (Joyner, 1992), with hydrogen ion appearing to be the predominant effector (Sinoway *et al.*, 1989; Victor *et al.*, 1988), although diprotonated phosphate has recently also been implicated (Sinoway *et al.*, 1994). Stimulation of these afferents results in an elevated mean arterial pressure achieved primarily via sympathetically mediated increases in systemic vasoconstriction, with elevations in heart rate playing a minor role (McCloskey and Mitchell, 1972; Rowell and O'Leary, 1990). It is thought that the primary role of such a pressure raising reflex is to correct a mismatch between blood flow and metabolism in ischemic exercising muscle (Rowell, 1997). For this to be the case, the elevations in SNA to exercising muscle which parallel those to resting muscle both in time course and magnitude (Hansen *et al.*, 1994) could not significantly affect the vascular bed of the exercising muscle mass. Evidence from dogs (O'Leary and Sheriff, 1995; Wyss *et al.*, 1983) and humans (Rowell *et al.*, 1991) supports this view, with elevations in blood pressure restoring approximately 50% of the blood flow error in ischemic muscle. Such a result is consistent with the concept of a functional sympatholysis, whereby local metabolic and flow dependent vasodilatory factors attenuate the effect of elevated sympathetic activity on vascular conductance in the exercising muscle (Eboute *et al.*, 1987; Rorie *et al.*, 1981; Laughlin *et al.*, 1996; Shepherd, 1983). However in humans, Joyner (1991) did not observe any restoration of blood flow in the exercising forearm made ischemic via 50 mmHg positive pressure, despite a progressive 20 mmHg increase in blood

pressure. He postulated that this was because of an increase in sympathetic activity to the exercising forearm muscles resulting in a vasoconstriction. This is consistent with the concept of a sympathetic restraint, whereby elevated sympathetic activity can reduce vascular conductance in an exercising muscle vascular bed (Laughlin *et al.*, 1996).

More recently, the chemoreflex effect on vascular conductance in non-ischemic exercising muscle has been investigated. Mittelstadt et al. (1994), in dogs running on a treadmill, found that terminal aortic occlusion severe enough to elicit an ~40 mmHg increase in blood pressure resulted in a forelimb vasoconstriction. However, the blood pressure elevation outweighed this constriction and forelimb blood flow was increased. Kagaya et al. (1994) observed that the addition of exhausting handgrip exercise in humans performing moderate, rhythmic calf plantar flexion resulted in an ~20 mmHg increase in blood pressure within ~50 sec and that exercising calf vasoconstriction contributed to this pressor response. In fact, the calf vasoconstriction outweighed the systemic blood pressure change such that exercising calf blood flow decreased. Both of these observations support the concept of a sympathetic restraint in non-ischemic, exercising muscle.

At present, no evidence exists concerning the *adaptation* of blood flow in non-ischemic muscle from rest to exercise under conditions of elevated SNA due to stimulation of the chemoreflex in another muscle group. The response would depend on the relative effects of increases in blood pressure vs. changes in muscle vascular conductance resulting from the competition between SNA and vasodilatory factors. Therefore, we used Doppler ultrasound to determine the forearm blood flow response in the non-ischemic, exercising forearm under two conditions: 1) a chemoreflex-mediated elevation in SNA established *prior to* the onset of forearm

exercise, and 2) a progressive increase in chemoreflex-mediated SNA *during* steady state exercise. We hypothesized that the elevated SNA would attenuate the blood flow adaptation from rest to exercise in experiment 1, and reduce the steady state exercise blood flow response in the exercising forearm in experiment 2.

METHODS

Subjects

Nine healthy, young male subjects (20.4 ± 0.7 yr, mean ± SE) volunteered for this study and gave written consent on a form approved by the Office of Human Research of the University after receiving full written and verbal details of the experimental protocol and any potential risks involved. Each subject came to the laboratory on 2 occasions, once for familiarization, and once to complete two trials in each of the 3 exercise protocols.

Experimental Design

Forearm exercise and chemoreflex stimulation. The subjects lay supine with their right arm supported in an extended position at an angle from the horizontal such that mid-forearm level was ~20 cm above heart level (Figure 5.1). Forearm exercise consisted of rhythmic, dynamic handgrip exercise at a contraction/relaxation duty cycle of 1-s/2-s performed in time with a signal light. The load was equivalent to 20% of the subject's maximal voluntary contraction (MVC) (9.4 ± 0.5 kg) determined from the strongest of three attempts prior to the experiment. This workrate resulted in an ~4-fold increase in forearm blood flow from rest to exercise, comparable with the calf blood flow response in the experiment of Kagaya et al. (1994).

A chemoreflex originating in the calf muscles was stimulated via rhythmic, plantar flexion exercise during calf circulatory occlusion (CE+O). Circulatory occlusion was achieved with the inflation of cuffs immediately distal to the knee to a supra-systolic pressure (250 mmHg) (see Figure 5.1). For condition A, the chemoreflex was maintained during forearm exercise by sustained calf circulatory occlusion, although calf exercise had ceased.

Experimental protocol. Figure 5.2 illustrates the 3 exercise protocols. Prior to the initiation of

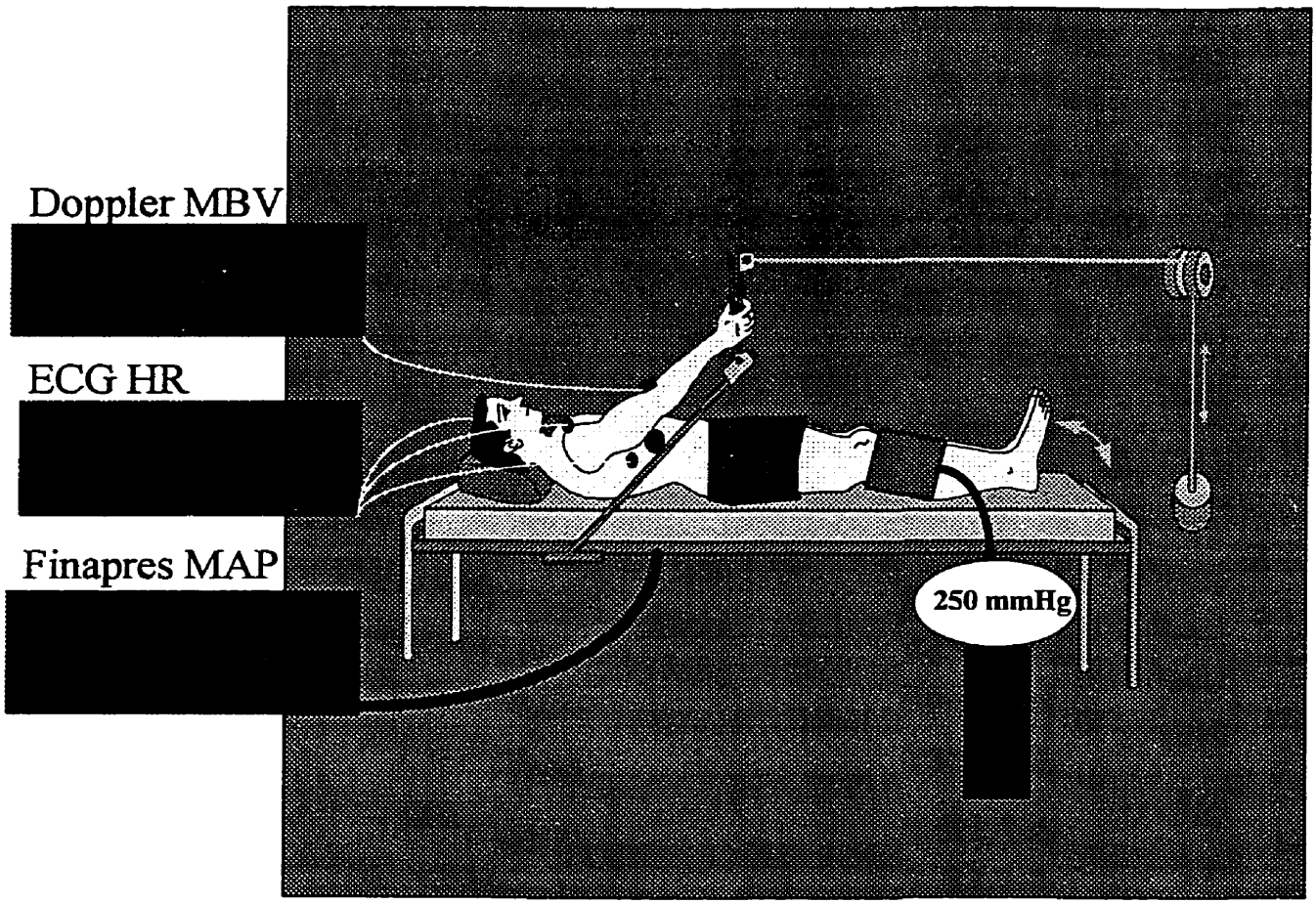

Figure 5.1 *Schematic depiction of the experimental setup for stimulation of the chemoreflex in the calf via rhythmic calf plantar flexion during calf circulatory occlusion (CE+O). Rhythmic forearm exercise was performed to raise and lower a weight. Finapres measures of mean arterial pressure were made on the contra lateral hand supported at heart level. Doppler ultrasound was recorded from the brachial artery.*

the experiments the subjects lay supine for 30-40 min and the forearm was cooled with a fan. This cooling was maintained throughout the experiments to minimize skin blood flow contributions to resting and exercising forearm blood flow. Condition A was used to evaluate the effect of a background chemoreflex-mediated elevation in SNA on the *adaptation* of forearm blood flow from rest to exercise. To accomplish this, CE+O was performed for 4 min. This resulted in a progressive increase in blood pressure. Pilot work had shown that this intervention could achieve a 20-25 mmHg increase in mean arterial pressure before subjects had to cease calf exercise due to discomfort. Therefore in all experiments CE+O was performed until mean arterial pressure had stabilized at 20-25 mmHg above resting levels, and thereafter the pressor response was maintained with continued calf circulatory occlusion. After 1 min of rest during calf circulatory occlusion, forearm exercise began and lasted for 5 min. At the end of forearm exercise, the calf occlusion cuffs were rapidly deflated and the recovery of cardiovascular responses was followed for 5 min.

Condition B was used to determine the effect of a progressive chemoreflex-mediated elevation in SNA on *steady state* exercising forearm blood flow. Forearm exercise was initiated under normal resting conditions. After 3 min of forearm exercise, CE+O began in combination with forearm exercise, with calf contractions being performed between forearm contractions. This resulted in a progressive elevation in mean arterial pressure over the next 6 min of forearm exercise, reaching a level similar to that induced in condition A. As in condition A, occlusion cuffs were immediately deflated at the end of forearm exercise. The responses of condition A and B were compared to condition C (control condition) which consisted of 9 min of forearm exercise only. Each subject performed the 3 exercise protocols in the same experimental session, with the

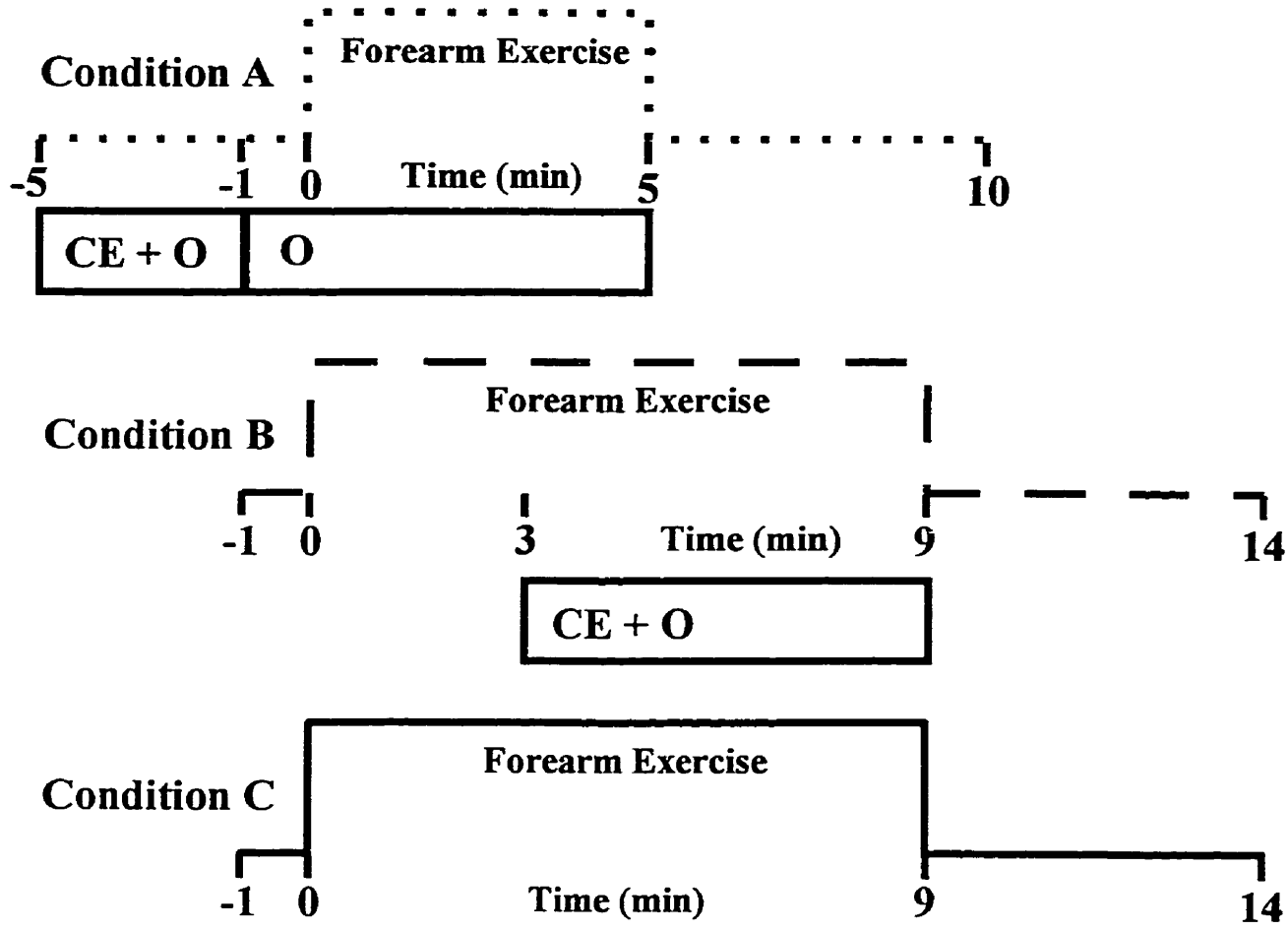

Figure 5.2 *Schematic depiction of the time course of the 3 experimental protocols, with lines coded to match data figures. Forearm exercise began at time = 0 min. Cardiovascular responses were monitored over a 1 min rest period, 5 (condition A, ...) or 9 (condition B, ——— and C, ———) min of forearm exercise and 5 min of recovery (all conditions). CE+O - calf exercise during calf circulatory occlusion. O - calf circulatory occlusion.*

order being randomized.

Data Acquisition

Heart rate (HR) and mean arterial pressure (MAP) were measured beat by beat. MAP was measured at heart level using a photoplethysmograph finger blood pressure cuff (Ohmeda 2300, Finapres, Lakewood, CO) on the middle finger of the left hand.

Forearm blood flow (FBF) was obtained beat by beat as the product of brachial artery mean blood velocity (MBV) and arterial cross sectional area:

$$\text{FBF (ml/min)} = \text{MBV (cm/s)} \bullet 60 \text{ s/min} \bullet \pi(\text{brachial artery diameter (cm)}/2)^2$$

Brachial artery blood velocity was measured with a 4-MHz pulsed Doppler ultrasound probe (Multigon Industries, model 500V, Mt. Vernon, NY) which was fixed to the skin over the brachial artery at the level of the antecubital fossa of the right elbow (Tschakovsky *et al.*, 1995). With this placement and arm position, probe insonation angle relative to the skin is 45° and the brachial artery is approximately parallel with the skin. Arterial cross-sectional area was measured by a separate, linear 7.5 MHz echo Doppler ultrasound probe operating in B mode (Toshiba model SSH-140A, Tochigi-Ken, Japan) simultaneously with pulsed Doppler measures of MBV. This probe was positioned ~9 cm proximal to the medial epicondyle, which was necessary to avoid acoustic interference between the probes. It has been shown previously in our laboratory that brachial artery diameters are not different between the two measurement sites (Shoemaker *et al.*, 1996). Imaged data were saved on video tape (Panasonic model AG-7300) for subsequent analysis. Arterial diameter was determined 4 times at rest and at 5 s, 10 s, 20 s, 30 s and thereafter every 30 s during forearm exercise and again at 5 s, 10 s, 20 s, 30 s and thereafter every 30 s of the 5 minute recovery period in each of the exercise conditions. Diameter measurements at

these times consisted of the average of 3 separate caliper measures of a frozen screen image of the brachial artery during diastole. All measurements were performed by the same operator.

Data Analysis

For each subject, the diameter data were fit with an exponential regression to reduce random measurement error and provide continuous diameter estimates for the beat by beat MBV to allow calculation of FBF. HR, MBV and MAP data were saved continuously at 100 Hz on a dedicated computer via analog-to-digital conversion. For analysis, the beat by beat data were averaged into 3-s bins corresponding to the contraction/relaxation duty cycle and then averaged across all subject trials to determine the mean response profile. For condition C, data from rest and the first 3 min of exercise in condition B are also part of the averaged response, since in effect this phase of condition B was identical to condition C. Mean values for HR, MAP and FBF reported at rest are the average of the 60-s rest period. Mean values at different times during forearm exercise are the average of 4 contraction/relaxation duty cycles for each subject (12 second average).

For estimates of forearm vascular conductance (FVC) the following procedure and rationale was applied. We and others (Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996) (for review, see Laughlin (1987)) have shown that the muscle pump can contribute to a change in blood flow through a vascular bed without a change in vascular conductance by expelling blood from the veins, thereby reducing the venous pressure and increasing the arterial - venous pressure gradient. Thus during dynamic exercise, using exercising muscle blood flow divided by arterial pressure can only provide an estimate of what has been termed "virtual conductance" (Sheriff *et al.*, 1993) representing both changes in resistance vessel caliber and the mechanical effect of muscle contraction. However, we have also shown that this mechanical effect of contraction does not

occur when the exercising muscle mass is well above heart level (see Figures 1 and 3 in Tschakovsky et al. (1996)) because the veins are virtually empty and little effective change in arterial - venous pressure gradient can be achieved by the mechanical effect of muscle contraction. Therefore if one were to determine the flow for one cardiac cycle during relaxation (which is unaffected by the compressive effects of contraction) divided by the arterial pressure, this would be expected to provide the best estimate of true vascular conductance. For this reason the forearm was elevated 20 cm above heart level for all experiments. At rest, FVC was calculated as the average FBF/MAP over the 60-s rest period. At 20 s, 30 s, 40 s, 1 min and thereafter every 30 s of exercise, FVC was calculated as the average of FBF measured over 3 separate beats during the relaxation phases between contractions divided by corresponding beat MAP.

Post exercise hyperemia was determined as the total forearm blood flow in excess of resting flow during the 5 min period of recovery following the cessation of forearm exercise.

Statistical Analysis

One way repeated measures analysis of variance was used to determine the effects of exercise condition on HR, MAP, FBF and FVC at rest and at different times during forearm exercise, and on the post exercise hyperemia. The level of significance for ANOVA was set at $P<0.05$, with significant differences further analyzed with Student-Newman-Keuls post hoc testing at time points where 3 conditions were being compared. All data are presented as means ± SE.

RESULTS

Adaptation from rest to exercise

CE+O prior to forearm exercise (Condition A) elevated heart rate compared to control (Condition C) (A 72.9 ± 5.8 vs. C 59.7 ± 3.3 beats/min, P=0.0028) and resulted in a 24% increase in MAP (A 122.5 ± 3.1 vs. C 98.5 ± 2.7 mmHg, P<0.0001). This indicated a strong activation of the muscle chemoreflex and suggested increased SNA activity (Figure 5.3). This effect was maintained by calf circulatory occlusion when calf exercise ceased, as evidenced by the continued similar elevation of HR and MAP in A vs. C (Figure 5.3). Resting FBF was not altered (P=0.877) by the increase in MAP since FVC was reduced by 24% (P=0.0352). That is, the chemoreflex-mediated increases in SNA vasoconstricted resistance vessels in the resting forearm muscles (see Figures 5.3 and 5.4).

With the start of forearm exercise FBF increased. This increase was markedly greater in A vs. C through the 5 min of exercise in A (Figure 5.3). The difference in FVC in A vs. C was abolished by 20 s of exercise (Figure 5.4). However, by 5 min of exercise, FVC in A vs. C was decreased by 16% (P=0.0018), although this did not compensate completely for the 25% elevation in MAP at this time such that FBF was still elevated in A vs. C (A 247.9 ± 15.0 vs. C 207.3 ± 9.4 ml/min, P=0.0197). In the control condition, only very minor changes in HR and MAP occurred during the course of exercise, indicating that forearm exercise per se was of moderate intensity and the forearm muscles were not ischemic.

Steady state exercise

FBF was relatively stable by 3 min of forearm exercise in the control condition. At this time in condition B, CE+O began. This resulted in an acute increase in HR but no immediate change in

MAP (Figure 5.3), suggesting that elevations in HR under these conditions did not significantly affect MAP. Progressive increases in MAP began after a delay of approximately 1 min and continued for the remaining 5 min of forearm exercise in condition B, eventually exceeding MAP in the control condition by 20% (B 129 ± 4 vs. C 107 ± 4 mmHg, P=0.0002) at the end of forearm exercise. Since FVC was not affected in condition B by the CE+O-induced progressive elevations in SNA (Figure 5.4), the gradual elevation in MAP resulted in proportional changes in FBF (Figure 5.3). HR, MAP and FBF responses by the end of condition B were not different from end exercise in condition A (B 77 ± 4 vs. A 77 ± 6 beats/min, P=0.954; B 129 ± 4 vs. A 132 ± 4 mmHg, P=0.138; B 266 ± 12 vs. A 247 ± 15 ml/min, P=0.105), suggesting the achievement of a similar stimulation of the chemoreflex in both conditions.

Post exercise recovery

Figure 5.5 illustrates the time course of recovery of HR, MAP and FBF following the end of exercise and the release of calf circulatory occlusion. In conditions A and B both HR and MAP display a large, rapid drop in the first 10-20 s of recovery, followed by a smaller progressive decrease towards baseline levels by 5 min. Little change is apparent in these variables following the cessation of forearm exercise in the control condition. The total FBF post exercise hyperemia was markedly reduced in A and B vs. C (A 121 ± 18 and B 182 ± 27 vs. C 309 ± 27 ml, P=0.001) with all three responses showing a similar, rapid decrease in flow over the first 10 s of recovery at which time the reduction in flow in condition C became markedly slowed. By the end of 5 min of recovery FBF was similar between conditions.

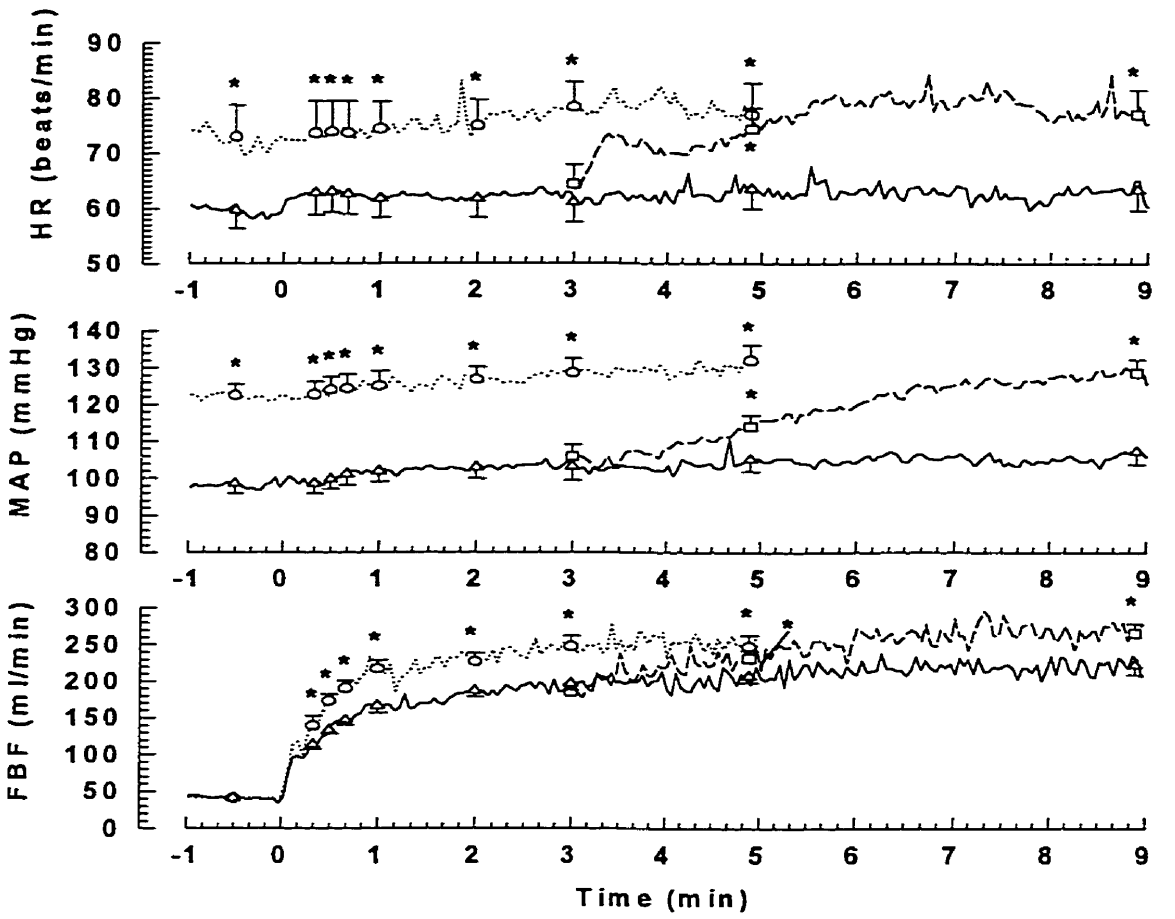

Figure 5.3 *This figure depicts the time course and magnitude of heart rate (HR), mean arterial pressure (MAP) and forearm blood flow (FBF) responses to 1) a transition from rest to forearm exercise under a background of chemoreflex-mediated elevation in SNA (condition A, ···o···) 2) the gradual addition of chemoreflex-mediated elevations in SNA starting at 3 min of forearm exercise (condition B, —□—) 3) forearm exercise without any chemoreflex-mediated elevations in SNA (condition C, —Δ —). Forearm exercise began at time = 0 min for all conditions and ended at time = 5 min for condition A and time = 9 min for condition B and C. Values at selected times with error bars give mean ±SE. * Indicates a significant difference from control (C) (P<0.05) at the corresponding time.*

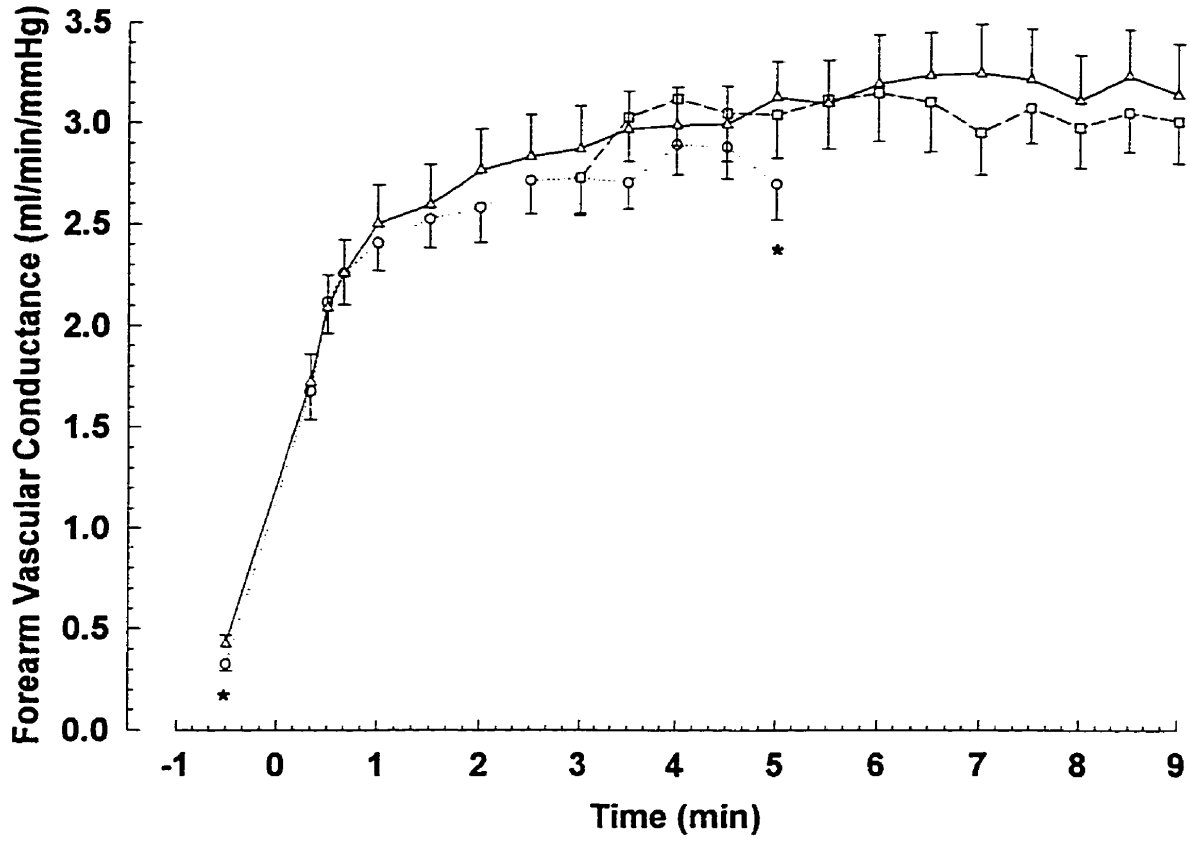

Figure 5.4 *This figure depicts the time course and magnitude of forearm vascular conductance (FVC) responses (see Methods: Data Analysis section for calculation of FVC). Symbols as in Figure 5.3. Forearm exercise began at time = 0 min for all conditions and ended at time = 5 min for condition A and time = 9 min for condition B and C. * Indicates significant difference from control (C) (P<0.05) at the corresponding time.*

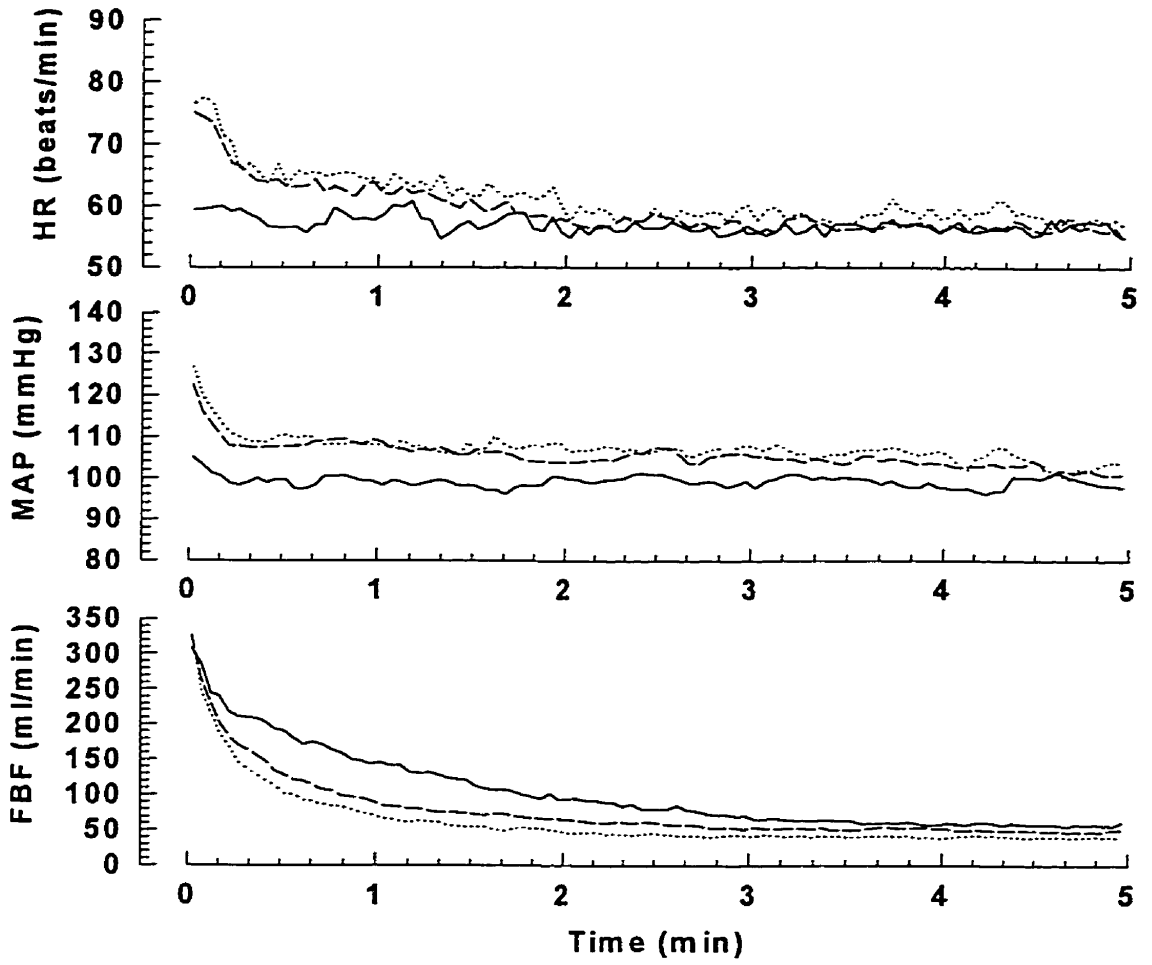

Figure 5.5 *The time course and magnitude of heart rate (HR), mean arterial pressure (MAP) and forearm blood flow (FBF) during 5 min of recovery. Symbols as in Figure 5.3 (see Results section for total post exercise hyperemia differences between conditions).*

DISCUSSION

The results of this study are in agreement with previous investigations which have identified that the muscle chemoreflex causes elevations in sympathetic nervous activity (SNA) leading to vasoconstriction in resting human limbs (Hansen *et al.*, 1994; Victor *et al.*, 1988; Joyner, 1992; Joyner and Wieling, 1993). However, the results do not support the working hypothesis that chemoreflex-mediated elevations in SNA would cause a vasoconstriction in the non-ischemic, *exercising* forearm. Rather, the forearm muscle vasoconstrictor effect was abolished with exercise, resulting in a passive elevation in exercising forearm blood flow due to elevations in systemic arterial pressure. This effect occurred both at the onset of a rest-to-exercise transition and when chemoreflex-mediated increases in SNA were progressively added during steady state forearm exercise and demonstrates the existence of a functional sympatholysis under the conditions of this study. Observations of a markedly reduced post exercise hyperemia following the passive elevation in blood flow during elevated SNA suggest that this elevation in blood flow had a positive impact on skeletal muscle metabolism.

Use of calf exercise during calf circulatory occlusion to elevate SNA

This study employed rhythmic calf exercise during calf circulatory occlusion (CE+O) followed by maintained calf circulatory occlusion in an attempt to create and maintain elevations in forearm sympathetic nervous activity. Since measurements of muscle sympathetic nervous activity (MSNA) in the forearm were not possible in this study, there was no direct evidence confirming that CE+O evoked an elevation in forearm sympathetic vasoconstrictor activity and that this was maintained with calf circulatory occlusion. However, numerous studies provide evidence that such a manipulation would consistently lead to elevated MSNA in resting (Hansen *et al.*, 1994;

Victor *et al.*, 1988; Joyner, 1992; Joyner and Wieling, 1993) and exercising (Mittelstadt *et al.*, 1994; Hansen *et al.*, 1994) muscle and that this would be maintained by circulatory occlusion (Joyner and Wieling, 1993; Hansen *et al.*, 1994; Joyner, 1992).

Presumably the muscle chemoreflex acts as a negative feedback reflex, whereby an inadequate oxygen delivery results in the accumulation of some substance(s) related to anaerobic metabolism which leads to a pressor response (Sheriff *et al.*, 1987) in an attempt to restore the blood flow to metabolism balance. Both hydrogen ion (Sinoway *et al.*, 1989; Victor *et al.*, 1988) and diprotonated phosphate (Sinoway *et al.*, 1994) have been implicated as primary affecters of a pressor response by their effects on muscle chemosensitive afferents and they would be expected to accumulate under conditions of reduced blood flow. In this study, CE+O would therefore have been expected to result in a strong stimulus for the muscle chemoreflex. While this would be expected to elevate SNA considerably, resultant elevations in arterial pressure would be sensed by carotid baroreceptors and the baroreflex would be expected to progressively oppose the chemoreflex (Mancia and Mark, 1983). This might explain the observation that the elevation in MAP tended to plateau at ~20-25 mmHg above control. Regardless, the magnitude by which MAP was elevated in this study was similar to that in other studies in which a significant effect on both resting and exercising muscle vascular conductance was observed (Saito *et al.*, 1990; Kagaya *et al.*, 1996; Kagaya *et al.*, 1994; Joyner, 1991). This, combined with the observation of a 24% reduction in forearm vascular conductance at rest, indicates that the CE+O intervention and maintained calf circulatory occlusion had a substantial effect on forearm MSNA.

Functional sympatholysis vs. sympathetic restraint

Original evidence for a functional sympatholysis stemmed from the observation that resistance

changes in response to sympathetic stimulation in an in situ dog preparation were attenuated in exercise (Remensnyder *et al.*, 1962; Kjellmer, 1965). Recently, this evidence has been dismissed as a mathematical artifact (Rowell, 1993) of the hyperbolic relationship of resistance to blood flow. However, a close look at Figure 5 from Remensnyder et al. (1962) supports the existence of a functional sympatholysis in exercising muscle. It illustrates systemic blood pressure and blood flow responses in a resting and an exercising dog limb to systemic arterial infusion of norepinephrine (NE). Infusion of NE elevated systemic pressure, resulting in an initial increase in flow to both the resting and exercising muscles prior to the NE entering those vascular beds. When NE entered the vascular bed of the resting muscle its blood flow decreased sharply back to resting levels prior to NE infusion while blood pressure continued to increase. This is consistent with a NE-induced vasoconstriction in this limb. However in the exercising limb the passive elevation in blood flow with increasing systemic arterial pressure was not interrupted when NE entered its vascular bed, indicating that no vasoconstrictor effect occurred in this limb. This response mirrors precisely the results of our study, where elevated SNA reduced resting vascular conductance but exercising forearm vascular conductance was not affected such that the elevated systemic arterial pressure resulted in proportional increases in exercising blood flow.

Thomas et al. (1994) also observed the same phenomenon of a passive elevation in exercising but not resting muscle blood flow with systemic elevations in arterial pressure induced by elevated SNA in rat gastrocnemius-plantaris (fast glycolytic) muscle at high frequencies of stimulation, but not in soleus muscle (slow oxidative). They interpreted these results to indicate that the sympatholytic effect required production of metabolites related to anaerobic metabolism. However, a sympatholysis can also be observed in dog muscle both in situ and running on a

treadmill, where the exercising muscle mass is predominantly oxidative. For example, Rowlands and Donald (1968) observed a much smaller % decrease in flow in response to sympathetic stimulation in exercise vs. rest in the dog hindlimb (contractions were electrically stimulated) under conditions of constant perfusion pressure (changes in flow are therefore directly proportional to vascular conductance), while Buckwalter and Clifford (1998) recently observed a reduction in the α_2-mediated vasoconstrictor effect with increasing dynamic exercise intensity in dogs running on a treadmill.

Numerous physiological mechanisms for a functional sympatholysis have been clearly documented. Inorganic phosphate, acetylcholine, adenosine, acidosis and potassium have all been demonstrated to inhibit sympathetic neuro-transmission (Eboute *et al.*, 1987; Rorie *et al.*, 1981; Verhaeghe *et al.*, 1977) (for review see (Shepherd, 1983; Shepherd and Vanhoutte, 1981)). Sympathetic vasoconstrictor effects are predominantly mediated by α_1 receptors in larger arterioles and by α_2 receptors (Ohyanagi *et al.*, 1991) in terminal arterioles. Anderson and Faber (1991) have shown that low frequency ($\leq$2 Hz) stimulation of rat cremaster muscle resulted in an attenuation of α_2-mediated constriction whereas higher frequency ($\geq$4Hz) stimulation reduced α_1 responsiveness and further attenuated α_2 constriction. Similar conclusions of a metabolic sensitivity to contractions specific to α_2 receptors can be drawn from the studies of Thomas et al. (1994) in rat gastrocnemius-plantaris muscle and Buckwalter and Clifford (1998) in dogs exercising on a treadmill. It has been suggested that control at this level of the arteriolar tree has minimal impact on vascular conductance (Rowell, 1997). However, observations of an α_2-mediated sympatholytic effect with significant impact on limb vascular conductance (Buckwalter and Clifford, 1998; Thomas *et al.*, 1994) argues against this suggesting instead that not only

distribution of blood flow, but also total blood flow, is affected by the metabolic sensitivity of α_2-mediated sympathetic constriction. Given the rapidity of the observed sympatholytic effect of exercise in this study, substances released early in exercise such as adenosine, potassium and acetylcholine would seem to be likely candidates.

Sympathetic restraint of muscle blood flow at rest in both animal (Thomas *et al.*, 1994; Remensnyder *et al.*, 1962; Thompson and Mohrman, 1983; Klabunde, 1986) and human (Hansen *et al.*, 1994; Victor *et al.*, 1988) models has been well documented, confirming the ability of the sympathetic constrictor nerves innervating the muscle vasculature to increase resting muscle vascular tone. Observations in this study of a 24% decrease in resting forearm vascular conductance under conditions of elevated SNA compared with control are consistent with this. Sympathetic control over resting tissues is obviously not confined to skeletal muscle, and in terms of contributing to a pressor response its ability to alter vascular conductance in other resting beds (splanchnic and renal) (Mittelstadt *et al.*, 1996; O'Hagan *et al.*, 1997; Rowell, 1993) is more important given the relatively small proportion of blood flow to skeletal muscle at rest (Rowell, 1993). Nevertheless, constriction of resting muscle clearly occurs.

In contrast, given its tremendous capacity to vasodilate (Rowell *et al.*, 1986), exercising skeletal muscle would appear to be a more appropriate target of sympathetic constriction in situations where blood pressure maintenance becomes crucial and the baroreflex is attempting to maintain the target systemic blood pressure (O'Leary *et al.*, 1997). Interestingly, such a phenomenon has been documented in studies both when the exercising muscle mass was large enough to challenge the pumping capacity of the heart (Secher *et al.*, 1977; O'Leary *et al.*, 1997) *and* in small muscle mass exercise where no threat to central circulatory limitations occur as long

as the exercise intensity of the second exercising muscle mass is large enough (Saito *et al.*, 1990; Kagaya *et al.*, 1996; Kagaya *et al.*, 1994). However, a number of studies have also observed that large increases in sympathetic activity to an exercising muscle mass induced by the addition of another exercising muscle mass did not alter its blood flow or vascular conductance (Savard *et al.*, 1989; Richardson *et al.*, 1995) and that when vascular conductance was reduced, it was only in proportion to the rise in arterial pressure, suggesting that a local autoregulation to prevent over-perfusion might be occurring as opposed to a sympathetic restraint (Richter *et al.*, 1992).

While the results of our study are qualitatively consistent with a number of other studies (Thomas *et al.*, 1994; Savard *et al.*, 1989; Richardson *et al.*, 1995; Sinoway *et al.*, 1989) employing different experimental protocols, they do not agree with the results of Kagaya et al. (1994; 1993) who used a similar exercise model in which supine subjects performed moderate calf plantar flexion exercise (10% MVC) and had exhaustive elbow flexion exercise (exhaustion within 50 seconds) superimposed (Kagaya *et al.*, 1994) or sustained isometric forearm handgrip at 30, 50 and 70% superimposed (Kagaya, 1993). In the former study, calf blood flow was elevated by ~4-fold from rest to exercise, similar to our forearm exercise response, and blood pressure increases due to elbow flexion were ~20-25 mmHg, also similar to our study. However, they observed a drop in exercising calf vascular conductance severe enough to significantly reduce exercising calf blood flow as measured by strain gauge plethysmography despite the increased arterial pressure. It is not clear why our results contrast, but it may be due to the magnitude of the sympathetic response induced in their study compared to ours, even though blood pressure elevation achieved was not different between the studies. Additionally, it may be a function of muscle fibre type, since the human soleus muscle is likely more oxidative than the forearm and

may therefore have reduced sympatholytic capacity (Thomas *et al.*, 1994).

Taken together, these apparent contradictions in the literature may reflect the complexity of the interaction between a given level of induced MSNA and the given metabolic vasodilatory environment in determining whether elevated MSNA is able to cause a vasoconstriction in the exercising muscle vascular bed. In other words, both functional sympatholysis and sympathetic restraint are robust phenomena, but they must interact with the baroreflex modulation of arterial pressure. At present, it is unclear what determines which of these dominates the vascular response in a given exercise condition.

Passive exercise hyperemia: speculation on its metabolic impact

A consistent effect of the passive elevation in blood flow to the exercising forearm induced by chemoreflex-mediated elevations in blood pressure was the observation of a reduced post-exercise hyperemia, regardless of whether the flow elevation occurred with the onset of exercise or was added progressively later in exercise. The observation of such a reduction in post-exercise hyperemia is consistent with a positive metabolic impact on the exercising non-ischemic forearm. We have demonstrated previously that elevated blood flow at the onset of forearm exercise allowed for a more rapid adaptation of aerobic metabolism with lower blood lactate (Hughson *et al.*, 1997), which would be expected to reduce reliance on PCr breakdown. Others have shown that increased supply of O_2 by hyperoxia after steady state exercise is reached allowed for partial re-synthesis of PCr (Haseler *et al.*, 1998). PCr recovery is directly related to post-exercise oxygen consumption following moderate exercise (Radda, 1996), and we would therefore expect that post-exercise hyperemia would be reduced if PCr depletion was less during exercise.

A reduced need for PCr re-synthesis provides a plausible explanation for *why* blood flow

returned to resting levels more rapidly following exercise under elevated flow conditions. However, it is not possible to identify mechanism(s) responsible for *how* this was achieved since this experiment was not designed to isolate such contributors. Therefore we cannot exclude the possibility that the reduced post-exercise hyperemia may not be directly related to muscle metabolism. For example, one contributor might be the baroreflex. Given that calf circulatory occlusion ceased immediately at the end of forearm exercise, calf vascular conductance would be near maximal at the onset of recovery. This might be expected to influence baroreflex control of blood pressure such that the more rapid vasoconstriction in the forearm during recovery in condition A and B was part of a baroreflex regulation of systemic blood pressure. Obviously, for this to be possible effective sympathetic constriction of the forearm would have to be re-established shortly after exercise ceased. Another potential contributor to the more rapid flow recovery following exercise during elevated blood flow might be a reduced interstitial concentration of vasodilatory metabolites responsible for the post exercise hyperemia, in essence a "washout" effect of the elevated exercising forearm blood flow. However, such a reduction was also likely to have occurred during exercise, yet had no apparent effect on exercising vascular conductance. Finally, it is not clear what role a myogenic response may have played, given that there was a sudden, rapid drop in systemic pressure at the end of forearm exercise when the calf occlusion cuffs were released.

Summary

Chemoreflex-mediated increases in SNA resulting from calf exercise during calf circulatory occlusion elevated mean arterial pressure by 24%. The elevation in MAP did not increase resting forearm blood flow due to a proportional reduction in forearm vascular conductance. This

forearm vasoconstriction was presumably part of the systemic sympathetic vasoconstrictor mechanism contributing to the well-documented pressor response elicited by the muscle chemoreflex (Rowell, 1997; Joyner, 1992; Rowell and O'Leary, 1990). However when forearm exercise was initiated, the chemoreflex-mediated effect on forearm vascular conductance was abolished. Likewise a gradual increase in SNA during steady state forearm exercise did not affect exercising forearm vascular conductance. In both cases forearm blood flow was elevated in proportion to blood pressure and the post-exercise hyperemia was substantially reduced, indicating a positive effect of this hyperemia on muscle metabolism.

There is clear evidence that exercising muscle is still under the influence of sympathetic vasoconstriction (O'Leary *et al.*, 1997) and the rationale that this vasoconstrictor influence must limit the metabolic vasodilation when the capacity of the exercising muscle mass approaches that of cardiac output (Rowell, 1993) is sound. However, there is equally clear evidence supporting the existence of a functional sympatholysis in exercising muscle (Buckwalter and Clifford, 1998; Thomas *et al.*, 1994; Remensnyder *et al.*, 1962), and physiological mechanisms that could account for this phenomenon have been clearly documented (Eboute *et al.*, 1987; Rorie *et al.*, 1981; Shepherd, 1983; Shepherd and Vanhoutte, 1981; Verhaeghe *et al.*, 1977). The observations of this study are best explained by the existence of a rapidly acting functional sympatholysis in the exercising forearm given the relative vasodilatory and SNA-mediated vasoconstrictor influences established by our exercise protocol. The rapidity of this sympatholysis suggests that sympatholytic affecters present early on in exercise (K^+, adenosine, acetylcholine) might be responsible for the initial effect. It remains to be determined exactly how sympathetic vasoconstriction, locally mediated vasodilation and sympatholytic mechanisms in exercising

muscle interact to determine whether a functional sympatholysis or a sympathetic restraint dominate the vascular response.

CHAPTER VI

General Discussion

Given that the compromise or enhancement of blood flow adaptation to exercise in humans has distinct metabolic and performance implications for exercising muscle (van Leeuwen *et al.*, 1992; Hughson *et al.*, 1997), it is of considerable interest to determine the mechanisms which can result in such influences on exercising muscle blood flow. While numerous techniques, both non-invasive (Williams *et al.*, 1978; Joyner *et al.*, 1990; Kowalchuk *et al.*, 1990) and invasive (Richardson *et al.*, 1995; Grassi *et al.*, 1996) have been applied to the measurement of exercising limb blood flow in humans, none possess the time resolution offered by Doppler ultrasound. Studies which have compared Doppler ultrasound with strain gauge plethysmography (Tschakovsky *et al.*, 1995; Levy *et al.*, 1979; Lubbers *et al.*, 1979; van Leeuwen *et al.*, 1992) and thermodilution (Radegran, 1997) have found good agreement. Our laboratory has conducted in vitro calibration of Doppler ultrasound in which porcine blood heated to 40° Celsius was pumped at a known flow rate through tygon tubing. Simultaneous measures of blood velocity with the 4 MHz pulsed Doppler probe (Shoemaker *et al.*, 1996) confirmed the validity of Doppler ultrasound measures of blood flow as performed in our laboratory. Doppler ultrasound has allowed us to obtain continuous measurements of blood flow during and after forearm exercise and limb position manipulation, thereby providing new information on the acute time course of blood flow changes and the likely involvement of regulatory mechanisms. The blood flow response with exercise is determined by the interaction of numerous factors which can affect the vascular conductance or the effective pressure gradient for blood flow (Delp and Laughlin, 1998; Shepherd, 1983). This thesis focused on the contribution of two such factors, reductions in

venous pressure and increases in sympathetic adrenergic activity, on the resting and exercising muscle blood flow response.

Role of Venous Pressure in Determining Forearm Blood Flow

Chapter II described a study designed to investigate the muscle pump contribution to early exercise hyperemia and used forearm cuff inflation as an analog of the compressive emptying of venous volume due to muscle contraction. When rhythmic cuff inflation was performed with the arm above heart level, no effect on arterial inflow occurred. However, when this was repeated with the arm below heart level, blood flow between cuff inflations increased. This indicated that compression of the vasculature per se did not elicit a vasodilation, and supported the interpretation that flow was likely elevated in the below heart condition due to an improvement in the arterial-venous pressure gradient. This evidence agrees with other work indicating that the muscle pump acts to reduce venous pressure and thereby improves the effective pressure gradient across the muscle vascular bed (Pollack and Wood, 1949; Folkow *et al.*, 1971; Sheriff *et al.*, 1993). It follows that the adaptation of blood flow to exercise should be enhanced in muscles that are exercising in the dependent position compared to above heart, since a greater initial hydrostatic column on the venous side would result in a greater potential increase in the local arterial-venous pressure gradient at the onset of contractions. In support of this, we have observed that the rate of increase in blood flow at the onset of exercise is faster when the exercising limb is in the dependent position (MacDonald *et al.*, 1998; Hughson *et al.*, 1997).

With regard to the control of the increase in blood flow at the onset of exercise, it was observed that mechanical venous emptying could not account for all of the rapid increase in blood flow following a single forearm contraction. This observation, combined with an analysis of the

characteristics of the blood flow response following cuff inflation vs. muscle contraction provides strong evidence that a vasodilatory mechanism exists which is capable of affecting muscle vascular conductance within 2 s of the first contraction of exercise.

Chapter III described a study designed to investigate blood flow responses to altered venous volume whereby elevation of the forearm above heart level was used to empty the veins. Venous pressure measured in a larger vein at the level of the antecubital fossa was obtained in a limited number of subjects, and indicated that arm elevation did lower venous pressure. Evidence from Chapter III indicated that venous volume reduction upon arm elevation above heart level resulted in an arteriolar vasodilation, albeit a transient one, initiated within a few seconds of venous emptying. When the arm was lowered during this transient dilation, the resultant hyperemia was greatly magnified compared to that observed when arm lowering occurred well after the transient vasodilation had disappeared. The transient vasodilation in the arm above heart position was abolished by maintaining venous volume with a congestion cuff about the upper arm. Likewise, the hyperemia upon lowering was also virtually abolished. This evidence suggests that the effect of emptying the veins might not be limited to alterations in the pressure gradient. It is consistent with the concept of a functional veno-arteriolar reflex observed by others (Nielsen *et al.*, 1988; Henriksen and Sejrsen, 1977; Henriksen *et al.*, 1983; Henriksen and Sejrsen, 1977), whereby venous volume changes can alter arterial vascular conductance via a local neural reflex. However, this study adds information on the function of this reflex in terms of the withdrawal of vasoconstriction upon limb elevation as opposed to initiation of vasoconstriction upon lowering of the limb as described by Henriksen and colleagues.

While these observations are in agreement with the basic tenet of the muscle pump, it is

difficult to reconcile them with other experiments in which direct measures of venous pressure in draining veins were made in animal models and no effect of altered venous pressure on arterial inflow was found (Naamani *et al.*, 1995; Magder, 1995; Braakman *et al.*, 1990; Magder, 1990). Those types of experiments consistently demonstrate the phenomenon of zero flow despite a positive arterial-venous pressure gradient (albeit their venous pressure measures are not immediately post-capillary). Additional evidence indicating that venous pressure might not have a direct impact on arterial inflow measured in a conduit artery can be seen in the lack of diastolic flow in resting muscle, despite diastolic pressures in excess of venous pressure (Tschakovsky *et al.*, 1996; Saupe *et al.*, 1995). It must be acknowledged therefore that the positive effect observed in studies in this thesis on arterial inflow when venous volume is reduced might not be directly related to venous pressure. If we examine the theoretical basis of both the vascular waterfall and the arterial compliance theories, then it is possible to speculate on an indirect effect of reduced venous volume and/or pressure on arterial inflow.

The vascular waterfall behaviour of the circulation depends on an external compressive force around a certain arteriolar region proximal to the capillaries and the collapsible nature of the vascular segment in question. This compressive force has as its contributors the smooth muscle tone of the arterioles (Permutt and Riley, 1963) and intuitively any other forces external to the blood vessels, such as interstitial pressure. Thus, internal vessel pressure must match this critical closing pressure (P_{crit}) or the vessel collapses. Therefore, the critical closing pressure acts as the effective back pressure to arterial inflow. Indeed, it has been demonstrated that reduction of smooth muscle tone as occurs during vasodilation reduces the critical closing pressure (Shrier and Magder, 1995). If interstitial pressure adds to the compressive force around the collapsible

arterioles, then a potential role for changes in venous volume becomes apparent. Muscles are sheathed in elastic connective tissue. Therefore alterations in venous volume would be expected to translate into alterations in interstitial pressure. In support of this, Radegran and Saltin (1998) have measured ~37% reduction in interstitial pressure in the deep quadriceps after a contraction when the leg was in the dependent position. Such an effect does not occur when the contracting muscle is above heart level (Jarvholm *et al.*, 1988). Furthermore, Shrier et al. (1997) provide evidence that the P_{crit} is affected by changes in interstitial pressure. It might therefore be possible that it is not venous pressure per se, but rather venous volume that impacts on arterial inflow by its contribution to P_{crit}.

A similar parallel can be drawn for the impact of venous volume-mediated changes in interstitial compressive forces in the arteriolar compliant compartment model. Any increase in external compressive forces would serve to increase the pressure within the arteriolar compliant region. If arterial inflow were determined by the back pressure of this region as suggested by some investigators (Spaan, 1985; Saupe *et al.*, 1995) and this back pressure were elevated by a compressive effect of surrounding venous volume, a reduction in flow would be predicted. Likewise, if the veins were emptied, thereby reducing this compression of the arteriolar compliance and reducing its pressure, an increase in arterial inflow would be expected.

Local Vasoconstrictor and Vasodilator Influences on Blood Flow

Chapter IV and V described studies designed to investigate the impact of increased systemic sympathetic nervous activity (SNA) on the blood flow adaptation to exercise in the forearm. In chapter IV, -60 mmHg LBNP was used to elevate SNA. With this level of LBNP, both cardiopulmonary and arterial baroreceptors are involved in the elevation of sympathetic outflow

(Abboud and Thames, 1983). Mean arterial pressure is usually preserved with this intervention, but pulse pressure is reduced (Tripathi *et al.*, 1989). In Chapter V, a chemoreflex initiated in the lower legs via calf exercise during calf circulatory occlusion elevated SNA. This intervention affects chemosensitive afferents in the calf muscles and elicits elevations in sympathetic outflow, with resultant increases in systemic blood pressure (Joyner, 1992). Based on previous observations that -60 mmHg LBNP reduced forearm blood flow (Shoemaker *et al.*, 1997; Strandell and Shepherd, 1967) and chemoreflex-induced elevations in SNA attenuated blood flow in non-ischemic exercising muscle (Kagaya, 1993; Kagaya *et al.*, 1994; Kagaya *et al.*, 1996; Mittelstadt *et al.*, 1994) it was hypothesized that elevations of SNA in these studies would impair the blood flow adaptation to forearm exercise. However, the results do not support this hypothesis. Rather, they suggest that in moderate small muscle mass exercise, local factors responsible for vasodilation rapidly blunt the effect of increased SNA as initiated by -60 mmHg LBNP and a calf muscle chemoreflex in our experiments, agreeing with other studies in humans and dogs which have indicated a functional sympatholysis in exercising muscle (Buckwalter and Clifford, 1998; Thomas *et al.*, 1994; Hansen *et al.*, 1996; Joyner *et al.*, 1990; Peterson *et al.*, 1988; Donald *et al.*, 1970; Remensnyder *et al.*, 1962).

Under conditions of LBNP, no effect on resting flow was observed. This was unexpected, since numerous studies have shown a clear compromise to resting blood flow when measured with strain gauge plethysmography (Strandell and Shepherd, 1967; Joyner *et al.*, 1990; Tripathi *et al.*, 1989). However, there were numerous differences in the conditions of our study which might account for this discrepancy. In a separate experiment, we observed that both strain gauge and Doppler measurements indicated a reduction in FBF in the arm above heart position. This

suggested that in our study where the arm was exercising below heart, the LBNP mediated reduction in resting venous volume (and therefore likely pressure) may have compensated for a reduced resting vascular conductance by improving the effective pressure gradient for FBF.

Further investigation was undertaken to compare the response to LBNP in a warm vs. cooled arm, since the subjects' arms had been cooled considerably in the experiment to minimize the contribution of skin blood flow, whereas this was not done in any previous experiments. It was observed that blood flow in the cool and warm arm was reduced immediately upon initiation of LBNP, but that it had recovered in the cool arm by 8 minutes. However, a similar response occurred in the warm arm. This might be interpreted to mean that cooling of the arm does not appear to explain the difference between the results of this experiment and those of others (Joyner *et al.*, 1990; Strandell and Shepherd, 1967; Tripathi *et al.*, 1989). However, it has been shown that cooling itself does result in a reflex delayed vasoconstriction in muscle (Mohan and Marshall, 1994; Thorsson *et al.*, 1985) and that cooling of tissue enhances α_2 vasoconstriction (Freedman *et al.*, 1992; Faber, 1988). Additionally, the phenomenon of sympathetic escape has been demonstrated with LBNP (Joyner *et al.*, 1990) and with sustained hypo perfusion (Lewis and Mellander, 1968). Sympathetic escape is simply a condition where vascular responsiveness to adrenergic stimulation is diminished over time despite the level of adrenergic stimulation being maintained. It is therefore possible that a combination of arm cooling to elevate resting forearm vasoconstriction and a sympathetic escape under maintained LBNP also contributed to the similar blood flow at rest in control vs. LBNP conditions.

One final possible explanation for the lack of reduction in resting forearm blood flow in LBNP, where forearm vasoconstriction would be expected, might be related to the elevated heart

rate. In this study, heart rate was elevated by ~25 beats/min in -60 mmHg LBNP. In comparison, Strandell and Shepherd (1967) observed only a 12-20 beats/min increase and Shoemaker et al. (1997) an 18 beat/min increase at -60 mmHg LBNP. This may have been due to the shorter period of LBNP prior to the start of data collection in these studies.

One argument for a heart rate effect has already been presented in Chapter IV. What follows here is an additional speculation on the combination of the conditions created by arm cooling in this experiment in combination with elevated heart rate. Tripathi and Nadel (1986) have provided evidence that suggests both skin and muscle blood flows are progressively reduced up to -20 mmHg LBNP, suggesting a vasoconstriction in both muscle and skin vascular beds. However, Vissing et al. (1994) did not observe any increases in skin sympathetic nerve discharge with such mild LBNP, although they did observe a reduction in skin blood flow when the arm was warm. There are no increases in heart rate associated with such low levels of LBNP (Rowell, 1993). If LBNP is progressively increased above -20 mmHg, little further reductions in muscle blood flow are observed, indicating that vasoconstriction of muscle is already near maximal at -20 mmHg (Tripathi and Nadel, 1986). However, skin blood flow continues to decrease with higher levels of LBNP, indicating that reductions in skin blood flow contribute to reductions in forearm blood flow with LBNP above -20 mmHg. In addition, heart rate increases progressively with LBNP above -20 mmHg (Rowell, 1993). If we were to progressively increase LBNP under the conditions of our study where forearm cooling likely maximized skin vasoconstriction prior to the onset of LBNP we might expect the following. With increases in LBNP up to -20 mmHg, a muscle vasoconstriction would occur, but no alteration in heart rate would occur. We would anticipate the observation of a reduced systolic pulse of blood into the forearm per beat in LBNP,

but no increase in the number of beats. Therefore, the reduced vascular conductance in LBNP would result in a reduced forearm blood flow. If we now progressively increased the level of LBNP, we would observe little further muscle vasoconstriction. However heart rate would begin to increase, reducing the diastolic period of zero arterial inflow. Thus, with increasing heart rate at higher levels of LBNP the reduction in forearm blood flow due to muscle vasoconstriction up to -20 mmHg might progressively be compensated for.

Once exercise began, it was observed that the initial, rapid increase in blood flow was attenuated in LBNP. However, blood flow quickly recovered during the second adaptation phase to mirror that in the control condition. Thereafter blood flow increased slightly over the next 3 minutes of forearm exercise in the control condition while remaining stable in LBNP, such that by the end of exercise blood flow was elevated by ~5-8% in control compared to LBNP. The reduced initial blood flow response to exercise could be due to elevated sympathetic drive to the forearm blunting the early increase in vascular conductance. Alternatively, since it has been shown that forearm volume is lowered with LBNP (Tripathi *et al.*, 1989), contraction may not have produced as great a change in the arterial-venous pressure gradient at the initiation of exercise in LBNP. Another factor to consider is the possible contribution of a veno-arteriolar reflex. Given that forearm venous volume would be reduced with LBNP, one would expect a release of the reflex vasoconstriction observed in a limb in the dependent position (Henriksen *et al.*, 1983; Henriksen and Sejrsen, 1977) prior to the initiation of exercise in LBNP. In control this contribution might not occur until the initiation of exercise when muscle contractions emptied the veins, thereby providing an additional mechanism for increasing vascular conductance early in exercise in the control condition that was not available in the LBNP condition.

In elevating SNA via a muscle chemoreflex, arterial blood pressure increased by 24%, however this was matched by a forearm vasoconstriction such that forearm blood flow at rest was not different. Upon initiation of exercise under this experimental condition, blood flow increased to a greater degree in the chemoreflex condition compared to control, and this difference was proportional to the difference in blood pressure such that calculated forearm vascular conductance during exercise was not different between conditions. Addition of a chemoreflex-mediated increase in SNA once steady state exercise had been reached resulted in a progressive increase in blood pressure, and a proportional increase in blood flow such that calculated forearm vascular conductance was not different from control. In both conditions where the elevated blood pressure effect of the chemoreflex served to augment exercising muscle blood flow, it was observed that the post exercise hyperemia was reduced compared to control. This is consistent with a positive metabolic effect of elevated exercising oxygen delivery on metabolism whereby PCr degradation is reduced (Haseler *et al.*, 1998).

An interesting contrast reveals itself between the observed effect on blood flow of an improved perfusion pressure gradient in this study induced by systemic elevations in pressure and that due to arm position in Chapter III. With systemic pressure elevations due to the chemoreflex, exercising blood flow was elevated over control. In Chapter III when arm position changed from below to above heart level, blood flow dropped immediately, indicating an effect of the local perfusion pressure gradient. Within seconds a partial restoration of flow occurred but it remained below levels observed during below heart exercise. This effect of posture has been observed previously (van Leeuwen *et al.*, 1992). When the arm was returned to the below heart position after 2 min, blood flow immediately overshot previous below heart steady state levels, but only

for a few seconds before returning to normal. This was despite the relative flow deficit that occurred over the past 2 min of exercise above heart. It is not clear why, under conditions in Chapter V, blood flow during exercise was allowed to exceed control conditions but under the experimental conditions in Chapter III it was not. This observation warrants further investigation.

Conclusions

This thesis attempted to identify i) whether alterations in venous pressure contributed to the effective ΔP across the vascular bed and therefore could impact on muscle blood flow at rest and during exercise and ii) whether elevations in systemic sympathetic nervous activity compromised exercising forearm muscle blood flow.

The results of the first two studies presented in this thesis provide new evidence in support of the muscle pump theory, indicating that mechanical or postural emptying of the forearm venous volume can effectively increase forearm blood flow. In addition, support for a veno-arteriolar reflex in which reductions in venous volume initiate an arterial vasodilation was found. Taken together, these data support the original hypothesis that a reduction in venous pressure can increase muscle blood flow. However, within the context of the muscle pump, it was identified that the immediate (0-5 s) increase in blood flow at the onset of exercise was not due solely to the mechanical effect of the muscle pump. Rather a rapid vasodilation detectible within two seconds of the first contraction of exercise must also contribute.

The results of the last two studies did not support the working hypothesis that elevated sympathetic nervous activity would compromise exercising muscle blood flow. Instead, they indicate the existence of a functional sympatholysis in the exercising forearm in the face of elevated sympathetic nervous activity. However, there is considerable disagreement in the

literature concerning this issue and there are as many studies that demonstrate a sympathetic restraint as there are that report a functional sympatholysis. For this reason, it is important to identify more clearly what determines which factor will dominate in the control of exercise blood flow under a given condition. This is one of a few issues addressed in the recommendations for future studies.

Future Considerations

This thesis examined factors related to the local pressure gradient across the vascular bed and the interaction of sympathetic vasoconstrictor influences with vasodilator influences on the response of vascular conductance. Measurements were predominantly non-invasive in nature. While this has advantages, determination of the precise nature of the mechanisms involved in the observed cardiovascular responses is problematic. The following is a list of recommendations specific to issues addressed by each paper.

1. Paper I (Chapter II) In this study, the effects of changes in venous pressure on muscle blood flow were examined, however venous pressure was either not measured directly and assumed to be affected by mechanical emptying and positioning of the arm above heart level or it was measured in a larger vein at the level of the elbow in a limited number of subjects (Paper II, Chapter III). It is currently not possible to obtain true measures of venous pressure at the post capillary level of the venules and it is this pressure that is likely of greatest importance if venous pressure does impact on blood flow. Given the inability to properly measure venous pressure, it is not clear how rapidly the venous pressure is restored during relaxation. This study examined the impact of venous emptying on flow at the onset of exercise. Here, vascular conductance is still low such that the absolute hyperemia induced by venous emptying is relatively small and restoration of venous pressure may take a few beats. The muscle pump hypothesis predicts that the same increase in arterial - venous pressure gradient at a greater vascular conductance (as would occur later in exercise) should result in a proportionally greater increase in flow. However, Naamani et al. (1995) observed virtually no effect of contractions on flow in maximally dilated dog gastrocnemius muscle, contrary to the prediction of the muscle pump hypothesis. A possible

explanation for this might be that, at high flows, the venous pressure is restored so rapidly that the existence of an improved pressure gradient is too brief to significantly improve blood flow. The likelihood of such an explanation is supported by the relatively low venous capacitance of muscle tissue (Magder, 1990). If this does in fact play a role, then one would expect that the magnitude of flow increase induced by mechanical emptying would not be proportionally elevated with increased vascular conductance and the significance of the muscle pump in exercise hyperemia during more intense exercise would be confined to the early changes in blood flow. Preliminary data in our laboratory has indicated that under conditions of near maximal vasodilation, brief cuff inflation around the forearm in the below heart position does not result in an increase in blood flow, supporting this hypothesis and warranting further investigation.

2. Paper II (Chapter III) The experimental model used in this study has numerous potential applications for understanding the control of the local muscle vasculature during rest and exercise. A transient hyperemia occurring shortly after the arm was elevated above heart level appeared to be mediated by the veno-arteriolar reflex. However, confirmation of this with the use of local neural blockade is necessary. In addition, the rapidity of adjustments in blood flow during exercise when the limb position relative to heart level is altered need to be explored in more detail. The observation of a rapid down regulation of the transient increase in blood flow when the arm is lowered below heart level after having been exercising with apparently reduced flow while above heart level suggests that hyper-perfusion of exercising muscle is prevented or minimized by factors influencing vascular conductance. Based on the rapidity of this response it would be hypothesized that it is not due to washout of vasodilator metabolites, but rather a reflex or myogenic response to changes in either arteriolar or perhaps venous pressures.

3. Paper III (Chapter IV) In this paper, a clear reduction in FBF was not found at rest with -60 mmHg LBNP. This is in contrast to numerous studies that have measured blood flow to the forearm using strain gauge plethysmography (Hansen *et al.*, 1996; Strandell and Shepherd, 1967; Tripathi and Nadel, 1986). Comparison of Doppler vs. strain gauge measures of blood flow in 5 subjects with the arm above heart level suggest a possible interaction of arm position relative to heart level with LBNP in determining resting forearm blood flow, possibly due to LBNP improving the local arterial-venous pressure gradient at rest. This effect however, may have contributed to the blunting of the initial rapid hyperemia since the gain in arterial-venous pressure gradient due to the onset of contractions would be reduced. Future studies are needed to determine if the effect of LBNP on forearm blood flow is such that at rest it improves the local arterial-venous pressure gradient with the limb in the dependent position, offsetting the sympathetic vasoconstrictor effect on vascular conductance, and at the initiation of exercise this same reduction in resting venous pressure compromises the gain in pressure gradient due to mechanical venous emptying with the first few contractions of exercise.

4. Paper IV (Chapter V) The fundamental issue raised by the results of this experiment and a review of the literature concerns the conditions under which either a sympathetic restraint or a functional sympatholysis dominates the local blood flow response during exercise. It may be that the fundamental determinant of whether exercising muscle vasculature responds to elevated sympathetic influences is the size of the exercising muscle mass, implicating a limitation of cardiac pumping capacity. Indeed, it has been stated that the function of a sympathetic restraint on blood flow is to prevent muscle vasodilation from outstripping the cardiac pumping capacity (Rowell, 1988). However, the effect is not limited to large muscle mass exercise. Likewise, in conditions

where large muscle mass exercise is performed there are examples of both sympathetic restraint and functional sympatholysis. It seems unlikely that a given sympathetic activation would have different effects on the vascular conductance in a given muscle simply because that muscle was exercising in combination with a number of other muscles as opposed to alone. Said differently, the local vascular responsiveness to sympathetic stimulation cannot be based on its "awareness" of whether other muscles are exercising and cardiac output limits are being reached and must rather be determined by local factors. Therefore, more experiments which gradually manipulate the muscle sympathetic nerve activity in a given exercising muscle mass or which gradually increase the amount of additional muscle mass exercising are needed to tease out the conditions which determine when the local increases in sympathetic activity actually dominate and limit exercising muscle blood flow.

A secondary recommendation is that of using this model of calf exercise during calf occlusion to elevate the blood flow response of the exercising forearm and, with the collection of venous blood samples, assess the impact of an improved blood flow response on muscle oxygen consumption and metabolism during and after an increase in workrate.

APPENDIX 1

Is the immediate post-exercise blood flow greater than during exercise due to removal of "sympathetic restraint" or simply the mechanical impedance of muscle contraction?

ABSTRACT

Mean forearm blood flow (FBF) during dynamic forearm exercise is lower than the immediate post-exercise hyperemia across a range of exercise intensities. This might be due to a mechanical interference of muscle contraction to flow during exercise which is removed when exercise ceases. Alternatively, there might be an immediate removal of SNS vasoconstrictor tone once exercise ceases, resulting in local vasodilatory factors causing an increase in limb vascular conductance. Doppler ultrasound techniques allow for a beat-by-beat assessment of limb blood flow, and thereby provide a means for assessing the role of a mechanical impedance of muscle contraction by separating flow during muscle relaxation from flow during muscle contraction. We reasoned that if vascular conductance was the same during relaxation phases in exercise compared to peak post-exercise levels, a mechanical effect of contraction could explain the greater immediate post-exercise hyperemia. Continuous measures of mean arterial pressure (Finapres) and brachial artery blood flow (Doppler) were made during and immediately after steady state dynamic forearm exercise at 25% and 75% maximal workrate. Peak between contraction blood flow (ml/min ± SE) and vascular conductance (ml/min/mmHg ± SE) were not different from peak post-exercise values for 25% (269.2 ± 17.3 vs. 245.6 ± 11.5 and 3.09 ± 0.27 vs. 2.89 ± 0.23) or 75% (609.7 ± 51.9 vs. 611.3 ± 61.4 and 5.76 ± 0.31 vs. 5.64 ± 0.49) conditions. This suggests that the immediate elevated post-exercise hyperemia above exercise flow levels is simply the result of removing the mechanical limitation to flow imposed by muscle contraction.

INTRODUCTION

At the end of rhythmic forearm exercise, the average blood flow increases above exercise levels (Shoemaker *et al.*, 1997). The increase is greater at higher exercise intensities. Given the observation that the degree of functional vasoconstriction in exercising dog muscle mediated by the sympathetic nervous system is elevated with exercise intensity (O'Leary *et al.*, 1997), this post exercise hyperemia might in part be mediated by the removal of a "sympathetic restraint" on blood flow when exercise ends. However, a number of studies have also indicated that a functional sympatholysis occurs in exercising muscle such that vasodilatory mechanisms effectively blunt the influence of sympathetic activity in the active muscle (Remensnyder *et al.*, 1962; Hansen *et al.*, 1996; Thomas *et al.*, 1994). Whether such a blunting is maintained following exercise is not known.

Blood flow during exercise is markedly elevated during the relaxation compared to contraction phase such that the average flow is less than that during relaxation (Walloe and Wesche, 1987; Kagaya and Ogita, 1992). Therefore, an alternative explanation to the removal of sympathetic restraint might be that the removal of the mechanical impedance to blood flow due to muscle contraction results in an elevated post exercise blood flow. In an attempt to distinguish which of these two mechanisms was responsible for the elevation in blood flow, we compared the blood flow and vascular conductance during beats between contractions with the peak beat flow and conductance immediately post-exercise. Beats between contractions were used to determine vascular conductance during exercise and avoid the effect of contraction on blood flow. It was reasoned that if the between-contraction peak vascular conductance was lower than the post-exercise peak vascular conductance, this would support the contention that a removal of

sympathetic restraint at the end of exercise was contributing to the greater post-exercise hyperaemia. If there was no difference, then the post-exercise hyperaemia could simply be explained by a removal of the impedance of muscle contraction on muscle blood flow.

METHODS

5 subjects participated in this study. Each performed a progressive rhythmic forearm exercise test to exhaustion (1 kg/min increase in workload, 1-s/ 2-s contraction/relaxation duty cycle) with the forearm above heart level. From this, the experimental workrates of 25% and 75% maximal workrate were determined. Subjects then came into the lab on a subsequent day and performed 1 trial of 5-min of forearm exercise in each of 25% and 75% maximal workrate conditions, with 25% trials always performed first to avoid fatigue effects from the 75% workrate. During the trials, forearm blood flow, mean arterial pressure at heart level and heart rate (see Chapter I for details) were recorded. The arm was supported by an armrest so that the mid forearm level was ~20-25 cm above heart level. In this position the veins were drained at rest, thereby minimizing the mechanical emptying of veins with muscle contraction during exercise (Tschakovsky *et al.*, 1996) which would increase the local arterial-venous pressure gradient on venous pressure. This allowed a comparison of calculated forearm vascular conductance between contractions vs. post-exercise. If the arm were below heart, then the local pressure gradient would be much different between contractions (veins squeezed empty by contraction, resulting in local pressure gradient equaling arterial pressure) vs. following exercise (veins refill, reducing local pressure gradient) making comparisons of vascular conductance based on arterial pressure problematic. Single beat flows during the last 30 s of exercise which occurred completely between contractions were averaged to provide the between contraction exercise blood flow and vascular conductance (beat flow/beat blood pressure). These were compared with the peak beat flow and conductance post-exercise. Statistical comparison between exercise and post exercise within each exercise condition was performed with one-way repeated measures analysis of variance (ANOVA). Where

multiple comparisons occurred as was the case with beat by beat blood pressure after exercise, further post hoc tests using Student-Neuman Keuls were done. $P<0.05$ was used to define statistical significance. All data are presented as means ± SE.

RESULTS

Figure A1.1 depicts the instantaneous beat-by-beat mean blood velocity (proportional to flow) in the last few seconds of exercise and the first few seconds of post-exercise hyperaemia. These raw data illustrate the effect of contraction on blood flow and shows that flow occurs predominantly between contractions, confirming the observations of Walloe and Wesche (1987) and Kagaya et al. (1992). Averaged blood flow (3-s average) responses are shown in Figure A1.2. Note the increase in blood flow post-exercise compared to the mean exercise flow, with the increase being much greater in the 75% condition. For the 75% condition, mean arterial pressure (MAP) increased throughout the 5 minutes of exercise (Figure A1.3). Heart rate increased in a step-wise fashion at the onset of exercise and thereafter remained fairly stable (Figure A1.4). The continued progressive increase in blood pressure in the 75% max WR condition was probably a function of a gradual sympathetically mediated systemic vasoconstriction: When exercise ended, MAP dropped rapidly (Figure A1.3). Part of this was likely due to the rapid decrease in HR, but the removal of systemic sympathetic vasoconstriction must also have contributed considerably. However this drop in MAP did not reach significance until the fifth beat following the end of exercise (Figure A1.5) (5^{th} beat 98.3 ± 5.3 vs. steady state 107.3 ± 5.4 mmHg), while the peak vascular conductance observed post-exercise occurred within the first 1-4 beats. Peak between contraction blood flow (ml/min ● SE) and vascular conductance (ml/min/mmHg ± SE) were not different from peak post-exercise values for 25% (269.2 ± 17.3 vs. 245.6 ± 11.5 and 3.09 ± 0.27 vs. 2.89 ± 0.23) or 75% (609.7 ± 51.9 vs. 611.3 ± 61.4 and 5.76 ± 0.31 vs. 5.64 ± 0.49) conditions (see Figure A1.6).

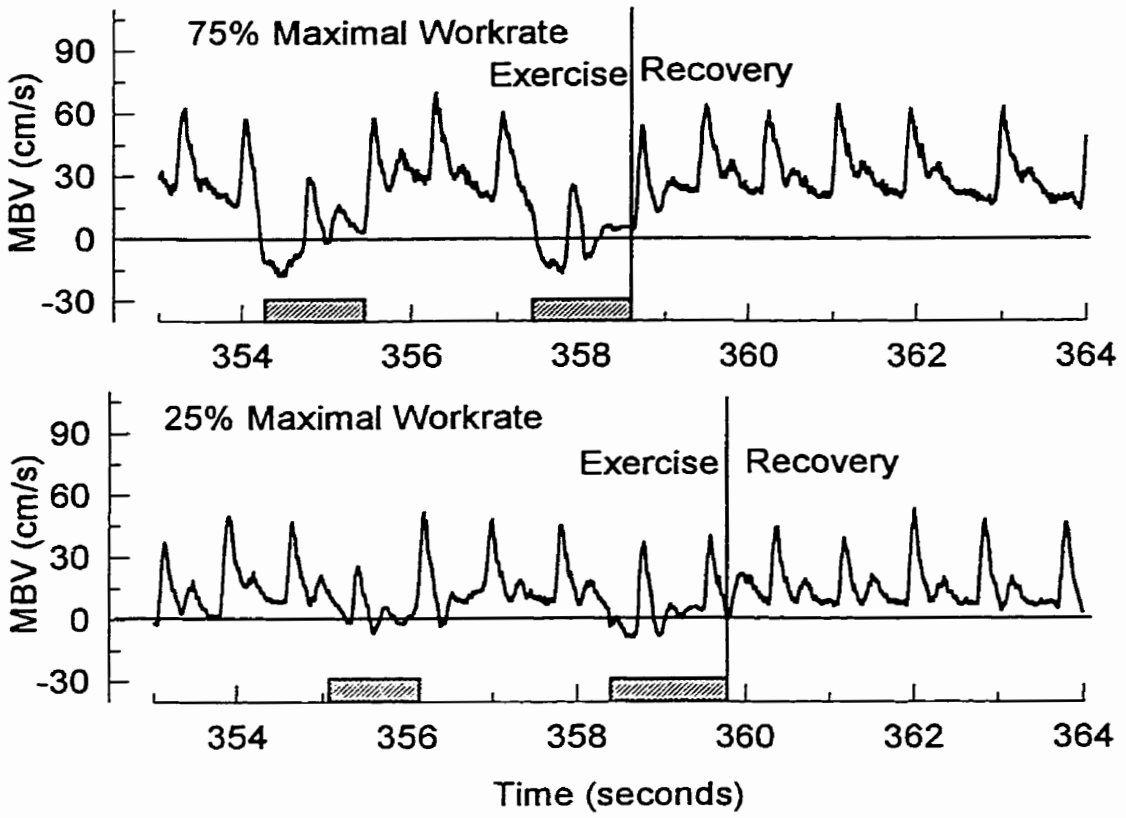

Figure A1.1 *Instantaneous mean blood velocity (MBV) for a single subject at 75% and 25% maximal workrates during the last few seconds of exercise and the first few seconds of recovery. The timing of muscle contractions is indicated by the hatched boxes. The effect of contraction on blood flow is evident, with most flow occurring between contractions. Peak post-exercise flow equaled flow occurring between contractions.*

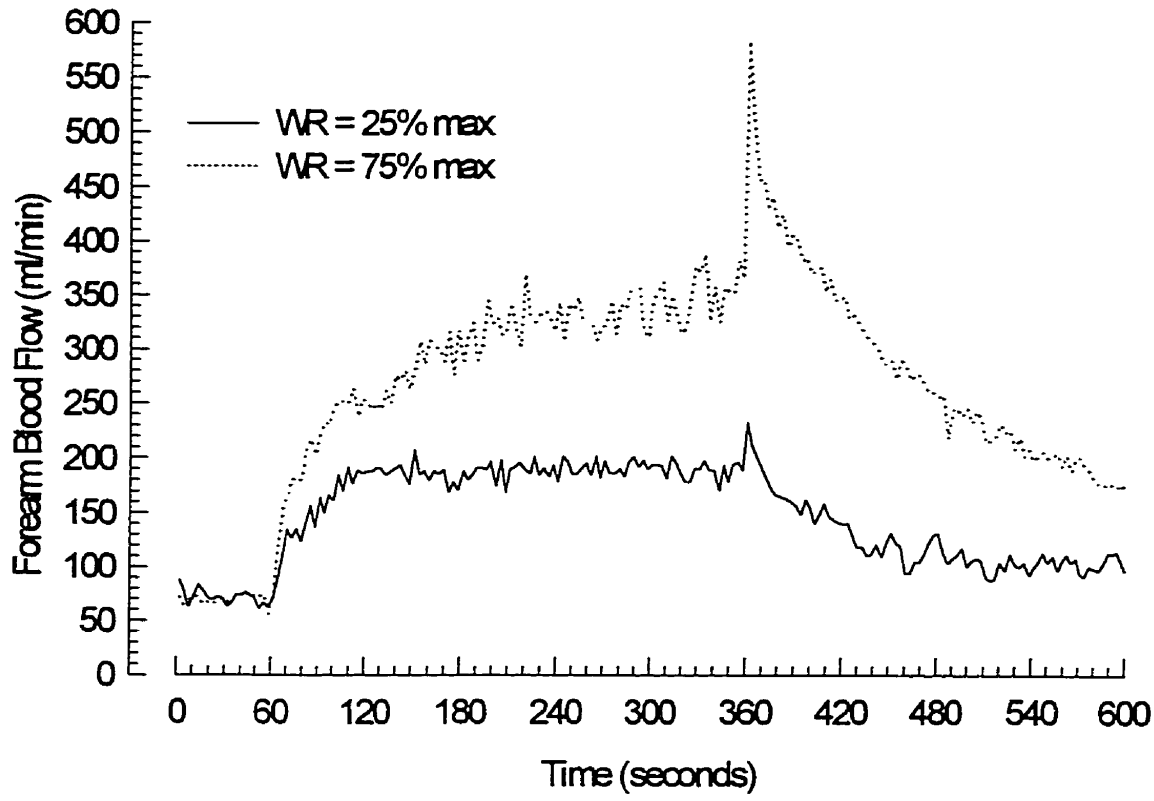

Figure A1.2 *Forearm blood flow (n=5) response at 75% and 25% maximal workrates. Exercise began at time = 60 s. Exercising blood flow is averaged over a complete duty cycle, reducing the contraction/relaxation variability evident in Figure A1.1. Blood flow increases in a biphasic manner, reaching a steady state early in the 25% workrate, but much later, if at all, in the 75% workrate. Note the large post exercise hyperemia in the 75% workrate condition which remains elevated above exercising flows for quite some time.*

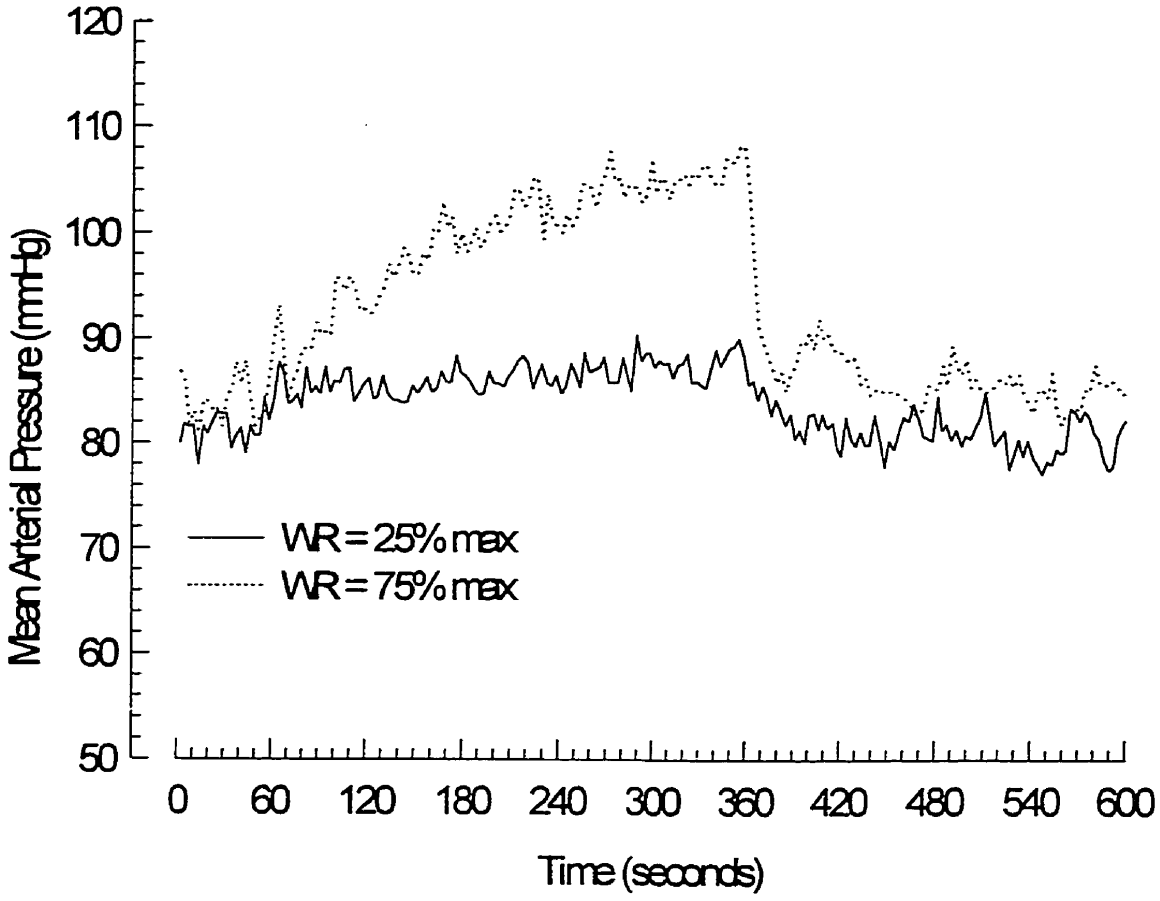

Figure A1.3 *Mean arterial pressure (MAP) response (n=5) at 25% and 75% workrates. Exercise began at time = 60 s. Note the progressive increase in MAP in the higher workrate and the rapid fall at the end of exercise (360 seconds).*

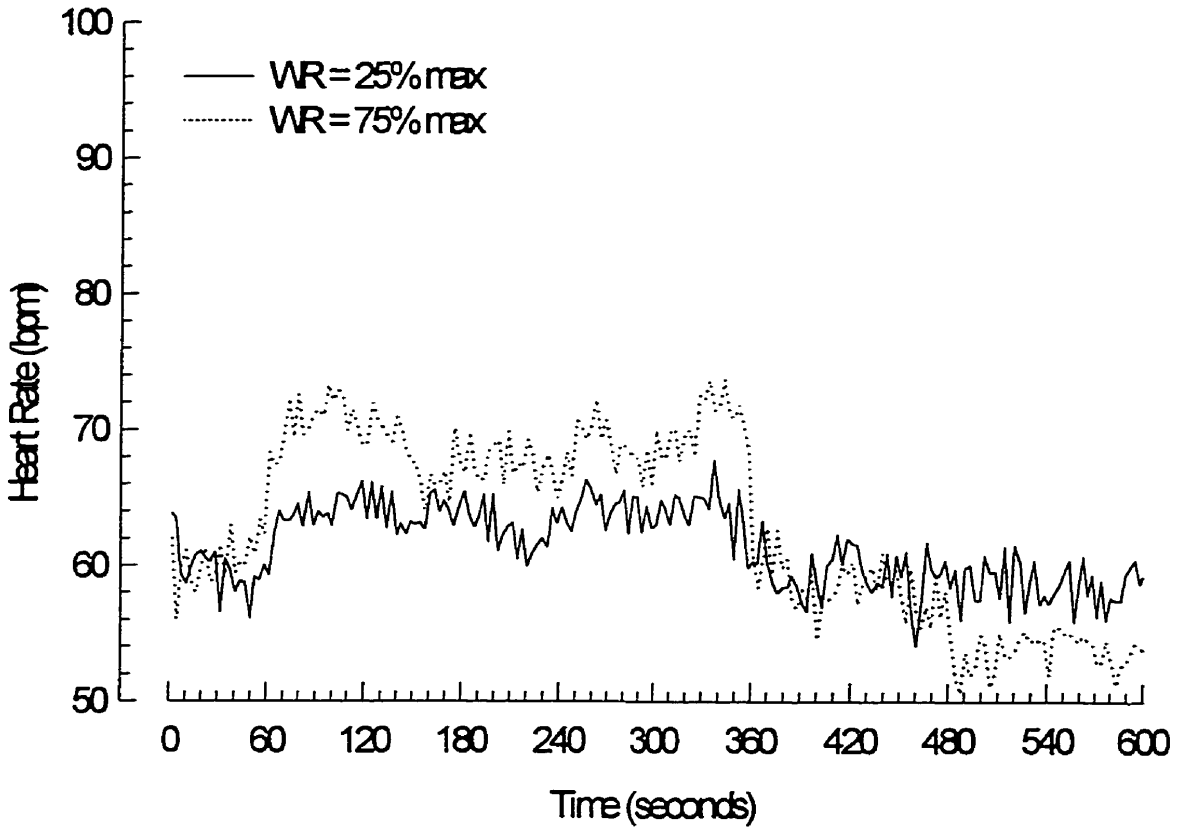

Figure A1.4 *Heart rate response (HR) (n=5) at 25% and 75% workrates. Heart rate increases immediately upon initiation of forearm exercise at time = 60 s, but then remains relatively constant.*

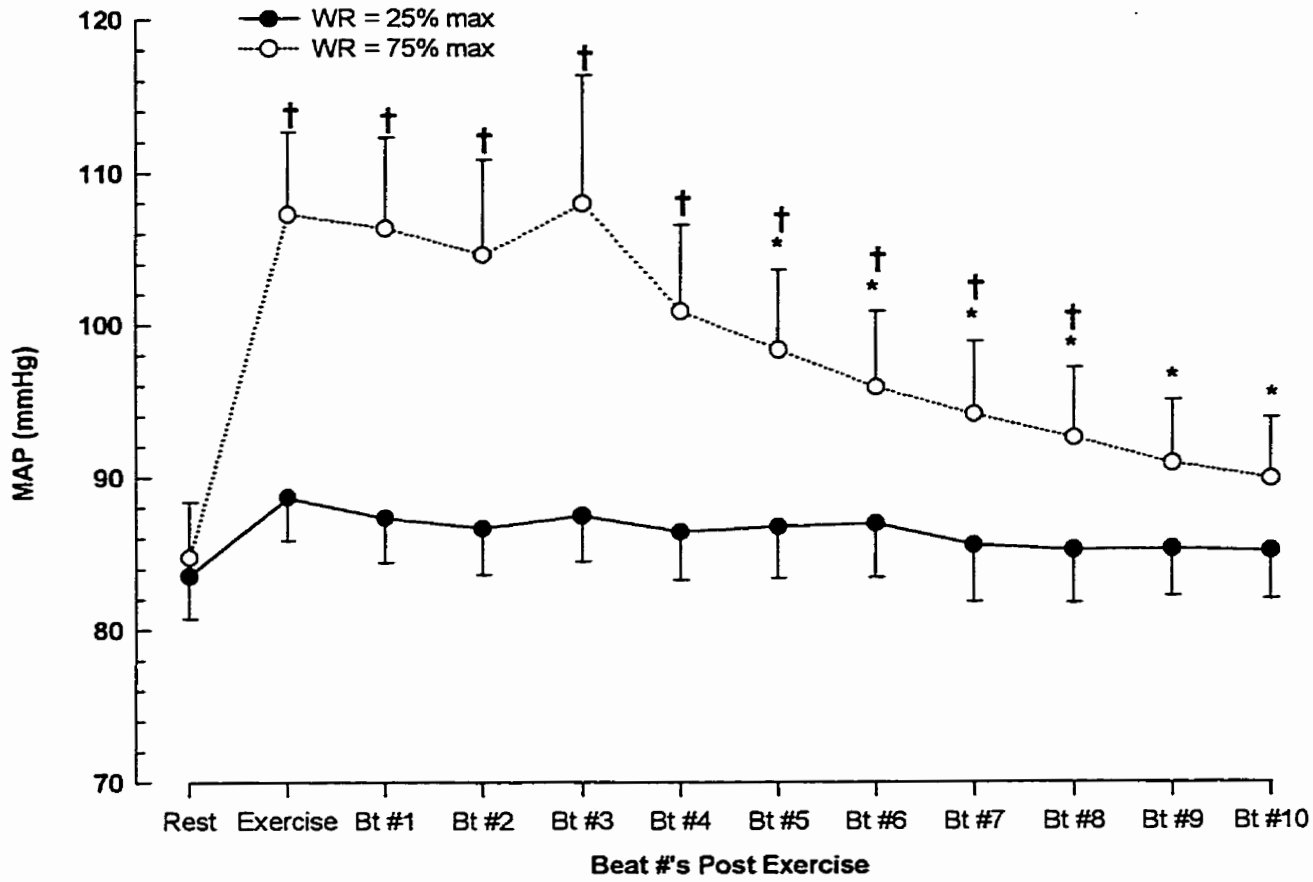

Figure A1.5 *Mean arterial pressure (MAP) at rest, steady state exercise and for consecutive beats following the end of exercise is shown for both the 25% and 75% workrates. * Significantly different from exercise for a given workrate. † Significantly different from rest for a given workrate.*

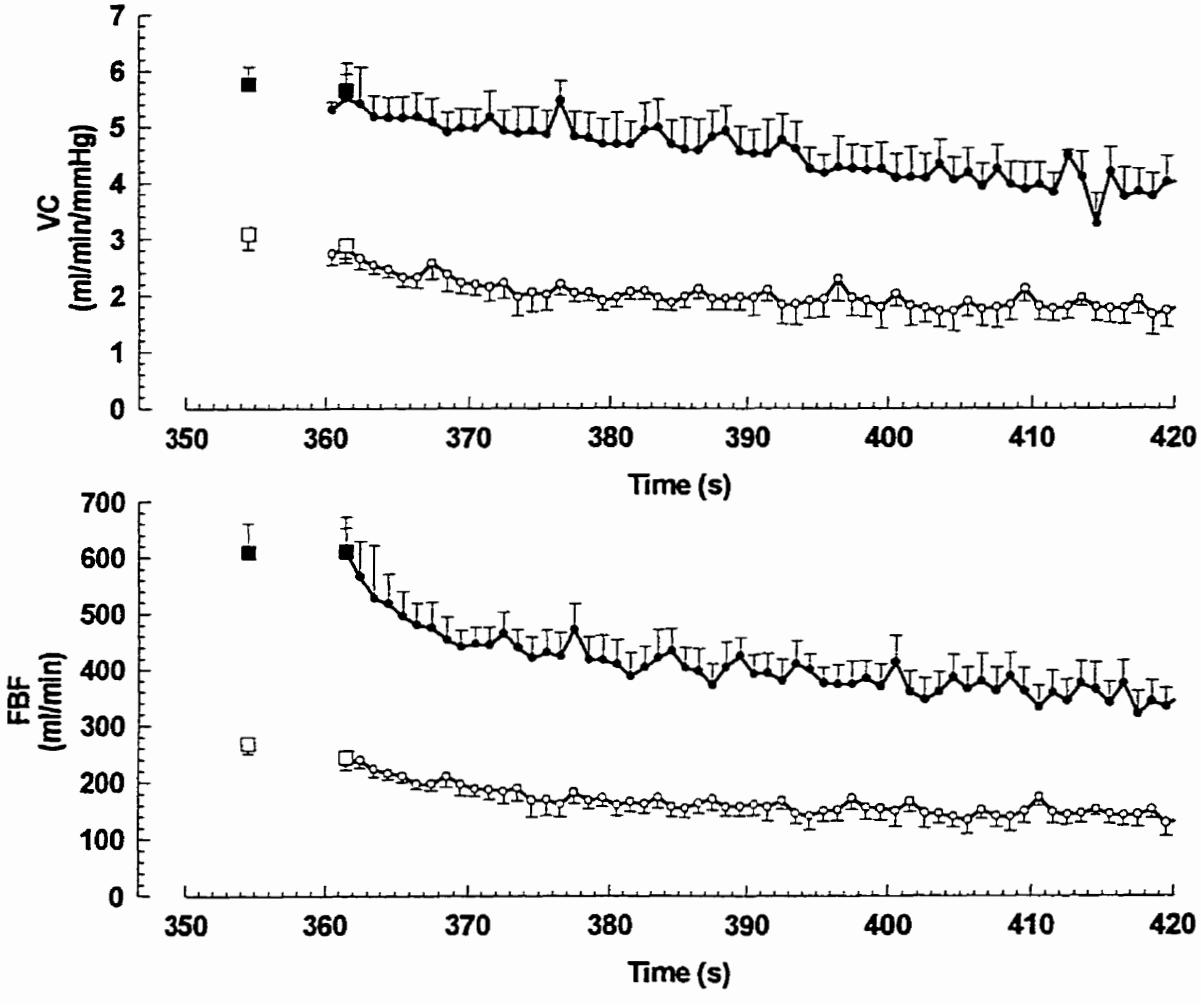

Figure A1.6 *Vascular conductance (VC) and forearm blood flow (FBF) is shown. Exercise ended at 360 s, therefore symbols at 355 s represent the between contraction VC and FBF for the 75% workrate (■) and the 25% workrate (□). The same symbols after 360 s represent the peak single beat VC and FBF during the post exercise hyperemia. 1 s interpolated VC and FBF data during the first minute after exercise ended are indicated by (●) for 75% workrate and (○) for 25% work rate. It can be seen here that the peak vascular conductance post exercise occurred in the first 2 seconds following the end of exercise.*

CONCLUSIONS

At the 75% max workrate, MAP by the end of 5 min of exercise was elevated by over 25%, and this effect was progressive, indicating that a systemic vasoconstriction was occurring since the change in heart rate occurred in a virtually step-wise manner at the beginning of the exercise bout. Blockade of α-adrenergic receptors has been shown to elevate exercising muscle blood flow in dogs (O'Leary *et al.*, 1997), suggesting that in dynamic exercise there is a functional sympathetic restraint of blood flow. It would follow then that the rapid removal of this sympathetic activity as occurs after exercise (Seals, 1989) might contribute to the degree of post exercise hyperemia.

If a withdrawal of sympathetic restraint in the forearm were to account for the elevated post exercise hyperemia relative to exercise, then it might be expected that the peak vascular conductance occurring post exercise would be greater than during exercise. Instead, there was no difference between peak post exercise vascular conductance and vascular conductance between contractions during the last 30 s of exercise. In this study, the peak post exercise vascular conductances occurred within the first 4 beats. Given that during exercise 2-s pauses occurred between contractions we would not expect a withdrawal of sympathetic activity to occur during the first 2 s after the conclusion of rhythmic contractions. In agreement with this, no changes in mean arterial blood pressure were measure with the Finapres during the first 4 beats following the end of exercise. These results indicate that, while there may be a rapid reduction in systemic sympathetic vasoconstriction, initiated within 4 beats following the end of 75% maximal rhythmic forearm exercise, the immediate increase in flow following the end of contractions can be explained by the removal of the mechanical impedance of blood flow by muscle contraction. In

addition, any delayed withdrawal of sympathetic vasoconstriction in the forearm that might have been present during exercise does not elevate forearm vascular conductance above peak levels occurring during and immediately after the end of exercise. However, it cannot be determined from the data whether or not a withdrawal of sympathetic restraint was in part responsible for the continued post exercise hyperaemia in this exercise model.

References

ABBOUD, F.M. AND THAMES, M.D. (1983). Interaction of cardiovascular reflexes in circulatory control. *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow.* chapt.19,(pp. 675-753). Bethesda, MD: Am. Physiol. Soc.

ANDERSEN, P., AND SALTIN, B. (1985). Maximal perfusion of skeletal muscle in human. *J Physiol (Lond)*, **366,** 233-249.

ANDERSON, K.M., AND FABER, J.E. (1991). Differential sensitivity of arteriolar alpha1- and alpha2-adrenoceptor constriction to metabolic inhibition during rat skeletal muscle contraction. *Circ Res,* **69,** 174-184.

ANREP, G.V., AND VON SAALFELD, E. (1935). The blood flow through the skeletal muscle in relation to its contraction. *J Physiol Lond,* **24,** 375-399.

ARDILL, B.L., BHATNAGAR, V.M., AND FENTEM, P.H. (1968). Observation of changes in volume of a congested limb as a means of studying the bahaviour of capacity vessels. *J Physiol,* **194,** 627-644.

BACCHUS, A., GAMBLE, G., ANDERSON, D., AND SCOTT, J. (1981). Role of the myogenic response in exercise hyperemia. *Microvasc Res,* **21,** 92-102.

BAILY, R.G., PROPHET, S.A., SHENBERGER, J.S., ZELIS, R., AND SINOWAY, L.I. (1990). Direct neurohumoral evidence for isolated sympathetic nervous system activation to skeletal muscle in response to cardiopulmonary baroreceptor unloading. *Circ Res,* **66,** 1720-1728.

BARENDSEN, G.J., AND VAN DEN BERG, J.W. (1984). Venous capacity, venous refill time and the effectiveness of the calf muscle pump in normal subjects. *Angiol,* 163-172.

BEVEGARD, B.S., AND SHEPHERD, J.T. (1966). Reaction in man of resistance and capacity

vessels in forearm and hand to leg exercise. *J Appl Physiol,* **21,** 123-132.

BORGSTROM, P., GRANDE, P.-O., AND LINDBOM, L. (1981). Responses of single arterioles in vivo in cat skeletal muscle to change in arterial pressure applied at different rates. *Acta Physiol Scand,* **113,** 207-212.

BRAAKMAN, R., SIPKEMA, P., AND WESTERHOF, N. (1990). Two zero-flow pressure intercepts exist in autoregulating isolated skeletal muscle. *Am J Physiol,* **258,** H1806-H1814.

BUCKWALTER J.B. AND CLIFFORD P.S. (1998).[Abstract] Reduced vascular responsiveness to alpha2-adrenergic agonist during dynamic exercise. *FASEB J,* 12, A691

CHEN, X., RAHMAN, A., AND FLORAS, J.S. (1995). Effects of forearm venous occlusion on peroneal muscle sympathetic nerve activity in healthy subjects. *Am J Cardiol,* **76,** 212-214.

CORCONDILAS, A., KOROXENEDIS, G.T., AND SHEPHERD, J.T. (1964). Effect of a brief contraction of forearm muscles on forearm blood flow. *J Appl Physiol,* **19,** 142-146.

CROSSLEY, R.J., GREENFIELD, A.D.M., PLASSARAS, G.C., AND STEPHENS, D. (1966). The interrelation of thermoregulatory and baroreceptor reflexes in the control of the blood vessels in the human forearm. *J Physiol Lond,* **183,** 628-636.

DELP, M.D., AND LAUGHLIN, M.H. (1998). Regulation of skeletal muscle perfusion during exercise. *Acta Physiol Scand,* **162,** 411-419.

DONALD, D.E., ROWLANDS, D.J., AND FERGUSON, D.A. (1970). Similarity of blood flow in the normal and the sympathectomized dog hind limb during graded exercise. *Circ Res,* **26,** 185-199.

DREW, G.M., AND WHITING, S.B. (1979). Evidence for two distinct types of postsynaptic alpha-adrenoreceptors in vascular smooth muscle in vivo. *Br J Pharmacol,* **67,** 207-215.

EBOUTE, Y., VANHOUTTE, P.M., AND SHEPHERD, J.T. (1987). Inorganic phosphate inhibits sympathetic neurotransmission in canine saphenous veins. *Am J Physiol,* **252,** H131-H134.

EHRLICH, W., BAER, R., BELLAMY, R., AND RANDOZZO, R. (1980). Instantaneous femoral artery pressure-flow relations in supine anesthetized dogs and the effect of unilateral elevation of femoral venous pressure. *Circ Res,* **47,** 88-98.

ELIA, M., AND KURPAD, A. (1993). What is the blood flow to resting human muscle? *Clin Sci,* **84,** 559-563.

ERIKSEN, M., WAALER, B.A., WALLOE, L., AND WESCHE, J. (1990). Dynamics and dimensions of cardiac output changes in humans at the onset and at the end of moderate rhythmic exercise. *J Physiol,* **426,** 423-437.

FABER, J.E. (1988). In situ analysis of alpha-adrenoceptors on arteriolar and venular smooth muscle in rat skeletal muscle microcirculation. *Circ Res,* **62,** 37-50.

FABER, J.E. (1988). Effect of local tissue cooling on microvascular smooth muscle and postjunctional alpha$_2$-adrenoreceptors. *Am J Physiol,* **255,** H121-H130.

FOLKOW, B. (1949). Intravascular pressure as a factor regulating the tone of the small vessels. *Acta Physiol Scand,* **17,** 289-310.

FOLKOW, B. (1952). A study of the factors influencing the tone of denervated blood vessels perfused at various pressures. *Acta Physiol Scand,* **27,** 99-117.

FOLKOW, B., GASKELL, P., AND WAALER, B.A. (1970). Blood flow through limb muscles during heavy rhythmic exercise. *Acta Physiol Scand,* **80,** 61-72.

FOLKOW, B., HAGLUND, U., JODAL, M., AND LUNDGREN, O. (1971). Blood flow in the

calf muscle of man during heavy rhythmic exercise. *Acta Physiol Scand,* **81,** 157-163.

FREEDMAN, R.R., SABHARWAL, S.C., MOTEN, M., AND MIGALY, P. (1992). Local temperature modulates alpha 1- and alpha 2-adrenergic vasoconstriction in men. *Am J Physiol,* **263,** H1197-H1200.

FRONEK, A. (1989). Plethysmography. In R. Moloney & G. Gavert (Eds.), *Noninvasive Diagnostics in Vascular Disease.* (pp. 11-40). New York: McGraw-Hill.

GASKELL, P., AND BURTON, A.C. (1953). Local postural vasomotor reflexes arising from the limb veins. *Circ Res,* **1,** 27-39.

GRASSI, B., POOLE, D.C., RICHARDSON, R.S., KNIGHT, D.R., ERICKSON, B.K., AND WAGNER, P.D. (1996). Muscle O_2 uptake kinetics in humans: implications for metabolic control. *J Appl Physiol,* **80,** 988-998.

HADDY, F.J., AND GILBERT, R.P. (1956). The relation of a venous-arteriolar reflex to transmural pressure and resistance in small and large systemic vessels. *Circ Res,* **4,** 25-32.

HANSEN, J., THOMAS, G.D., HARRIS, S.A., PARSONS, W.J., AND VICTOR, R.G. (1996). Differential sympathetic neural control of oxygenation in resting and exercising human skeletal muscle. *J Clin Invest,* **98,** 584-596.

HANSEN, J., THOMAS, G.D., JACOBSEN, T.N., AND VICTOR, R.G. (1994). Muscle metaboreflex triggers parallel sympathetic activation in exercising and resting human skeletal muscle. *Am J Physiol,* **266,** H2508-H2514.

HASELER LJ, RICHARDSON RS, AND HOGAN MC. (1998).[In Press] Phosphocreatine hydrolysis during submaximal exercise: the effect of FIO_2. *J Appl Physiol*

HENRIKSEN, O. (1991). Sympathetic reflex control of blood flow in human peripheral tissues.

Acta Physiol Scand, **143,** 33-39.

HENRIKSEN, O., AMTORP, O., FARIS, I., AND AGERSKOV, K. (1983). Evidence for a local sympathetic venoarterioloar "reflex" in the dog hindlimb. *Circ Res,* **52,** 534-542.

HENRIKSEN, O., AND SEJRSEN, P. (1977). Local reflex in microcirculation in human skeletal muscle. *Acta Physiol Scand,* **99,** 19-26.

HENRIKSEN, O., AND SEJRSEN, P. (1977). Effect of "vein pump" activation upon venous pressure and blood flow in human subcutaneous tissue. *Acta Physiol Scand,* **100,** 14-21.

HENRIKSEN, O., SKAGEN, K., HAXHOLDT, O., AND DYRBERG, V. (1983). Contribution of local blood flow regulation mechanisms to the maintenance of arterial pressure in upright position during epidural blockade. *Acta Physiol Scand,* **118,** 271-280.

HIATT, W.R., HUANG, S.Y., REGENSTEINER, J.G., MICCO, A.J., ISHIMOTO, G., MANCO-JOHNSON, M., DROSE, J., AND REEVES, J. (1989). Venous occlusion plethysmography reduces arterial diameter and flow velocity. *J Appl Physiol,* **66,** 2239-2244.

HILDEBRANDT, W., HERRMANN, J., AND STEGEMANN, J. (1993). Vascular adjustment and fluid reabsorption in the human forearm during elevation. *Eur J Appl Physiol,* **66,** 397-404.

HILDEBRANDT, W., HERRMANN, J., AND STEGEMANN, J. (1994). Fluid balance versus blood flow autoregulation in the elevated human limb: the role of venous collapse. *Eur J Appl Physiol,* **69,** 127-131.

HOCHACHKA, P.W., AND MATHESON, G.O. (1992). Regulating ATP turnover rates over broad dynamic work ranges in skeletal muscles. *J Appl Physiol,* **73,** 1697-1703.

HOGAN, M.C., GLADDEN, L.B., GRASSI, B., STARY, C.M., AND SAMAJA, M. (1998). Bioenergetics of contracting skeletal muscle after partial reduction of blood flow. *J Appl Physiol,*

84, 1882-1888.

HUGHSON, R.L., SHOEMAKER, J.K., TSCHAKOVSKY, M.E., AND KOWALCHUK, J.M. (1997). Dependence of muscle VO_2 on blood flow dynamics at onset of forearm exercise. *J Appl Physiol,* **81,** 1619-1626.

IMHOLZ, B.P.M., SETTELS, J.J., VAN DER MEIRACKER, A.H., WESSELING, K.H., AND WIELING, W. (1990). Non-invasive continuous finger blood pressure measurement during orthostatic stress compared to intra-arterial pressure. *Cardiovasc Res,* **24,** 214-221.

JACKMAN, A.P., AND GREEN, J.F. (1990). A theoretical description of arterial pressure-flow relationships with verification in the isolated hindlimb of the dog. *Ann Biomed Eng,* **18,** 89-101.

JARVHOLM, U., STYF, J., SUURKALA, M., AND HERBERTS, P. (1988). Intramuscular pressure and muscle blood flow in supraspinatus. *Eur J Appl Physiol,* **58,** 219-224.

JOHNSON, P.C., AND INTAGLIANETTA, M. (1976). Contributions of pressure and flow sensitivity to autoregulation in mesenteric arterioles. *Am J Physiol,* **231,** 1686-1698.

JONES, R.D., AND BERNE, R.M. (1965). Evidence for a metabolic mechanism in autoregulation of blood flow in skeletal muscle. *Circ Res,* **17,** 540-554.

JOURNO, H.J., CHANUDET, X.A., PANNIER, B.M., LAROQUE, P.L., LONDON, G.M., AND SAFAR, M.E. (1992). Hysteresis of the venous pressure-volume relationship in the forearm of borderline hypertensive subjects. *Clin Sci,* **82,** 329-334.

JOYNER, M.J. (1991). Does the pressor response to ischemic exercise improve blood flow to contracting muscles in humans? *J Appl Physiol,* **71,** 1496-1501.

JOYNER, M.J. (1992). Muscle chemoreflexes and exercise in humans. *Clin Auto Res,* **2,** 201-208.

JOYNER, M.J., LENNON, R.L., WEDEL, D.J., ROSE, S.J., AND SHEPHERD, J.T. (1990). Blood flow to contracting human muscles: influence of increased sympathetic activity. *J Appl Physiol,* **68,** 1453-1457.

JOYNER, M.J., NAUSS, L.A., WARNER, M.A., AND WARNER, D.O. (1992). Sympathetic modulation of blood flow and O_2 uptake in rhythmically contracting human forearm muscles. *Am J Physiol,* **263,** H1078-H1083.

JOYNER, M.J., SHEPHERD, J.T., AND SEALS, D.R. (1990). Sustained increases in sympathetic outflow during prolonged lower body negative pressure in humans. *J Appl Physiol,* **68,** 1004-1009.

JOYNER, M.J., AND WIELING, W. (1993). Increased muscle perfusion reduces muscle sympathetic nerve activity during handgripping. *J Appl Physiol,* **75,** 2450-2455.

KAGAYA, A. (1993). Relative contraction force producing a reduction in calf blood flow by superimposing forearm exercise on lower leg exercise. *Eur J Appl Physiol,* **66,** 309-314.

KAGAYA, A., AND OGITA, F. (1992). Blood flow during muscle contraction and relaxation in rhythmic exercise at different intensities. *Ann Physiol Anthrop,* **11,** 251-256.

KAGAYA, A., OGITA, F., AND KOYAMA, A. (1996). Vasoconstriction in active calf persists after discontinuation of combined exercise with high-intensity elbow flexion. *Acta Physiol Scand,* **157,** 85-92.

KAGAYA, A., SAITO, M., OGITA, F., AND SHINOHARA, M. (1994). Exhausting handgrip exercise reduces the blood flow in the active calf muscle exercising at low intensity. *Eur J Appl Physiol,* **68,** 252-257.

KAUFMAN, M.P. AND FORSTER, H.V. (1996). Reflexes controlling circulatory, ventilatory

and airway responses to exercise. In L.B. Rowell & J.T. Shepherd (Eds.), *Handbook of Physiology.* chapt.10,(pp. 381-447). New York: American Physiological Society.

KJELLMER, I. (1965). On the competition between metabolic vasodilatation and neurogenic vasoconstriction in skeletal muscle. *Acta Physiol Scand,* **63,** 450-459.

KLABUNDE, R.E. (1986). Attenuation of reactive and active hyperemia by sympathetic stimulation in dog gracilis muscle. *Am J Physiol,* **251,** H1183-H1187.

KNIGHT, D.R., SCHAFFARTZIK, W., POOLE, D.C., HOGAN, M.C., BEBOUT, D.E., AND WAGNER, P.D. (1993). Effects of hyperoxia on maximal leg O2 supply and utilization in men. *J Appl Physiol,* **75,** 2586-2594.

KOWALCHUK, J.M., KLEIN, C.S., AND HUGHSON, R.L. (1990). The effect of beta-adrenergic blockade on leg blood flow with repeated maximal contractions if the triceps surae muscle group in man. *Eur J Appl Physiol,* **60,** 360-364.

LAUGHLIN, M.H. (1987). Skeletal muscle blood flow capacity: the role of the muscle pump in exercise hyperemia. *Am J Physiol,* **253,** H296-H306.

LAUGHLIN, M.H., KORTHUIS, R.J., DUNCKER, D.J., ET AL. (1996). Control of blood flow to cardiac and skeletal muscle during exercise. In L.B. Rowell & J.T. Shepherd (Eds.), *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems. sect. 12.* chapt.16,(pp. 705-769). New York: Oxford University Press.

LEVY, B.I., VALLADARES, W.R., GHAEM, A., AND MARTINEAUD, J.P. (1979). Comparison of plethysmographic methods with pulsed Doppler blood flowmetry. *Am J Physiol,* **236,** H899-H903.

LEWIS, D.H., AND MELLANDER, S. (1968). Competitive effects of sympathetic control and

tissue metabolites on resistance and capacitance vessels and capillary filtration in skeletal muscle. *Acta Physiol Scand,* **56,** 162-188.

LEWIS, S.F., TAYLOR, W.F., GRAHAM, R.M., PETTINGER, W.A., SCHUTTE, J.E., AND BLOMQVIST, C.G. (1983). Cardiovascular responses to exercise as functions of absolute and relative work load. *J Appl Physiol (Respirat Environ Exercise Physiol),* **54,** 1314-1323.

LEYK, D., EßFELD, D., BAUM, K., AND STEGEMANN, J. (1994). Early leg blood flow adjustment during dynamic foot plantarflexions in upright and supine body position. *Int J Sports Med,* **15,** 447-452.

LIND, A.R., AND WILLIAMS, C.A. (1979). The control of blood flow through human forearm muscles following brief isometric contractions. *J Physiol,* **288,** 529-547.

LUBBERS, J., BERNINK, P.J.L.M., BARENDSEN, G.J., AND VAN DEN BERG, J.W. (1979). A continuous wave doppler velocimeter for monitoring blood flow in the popliteal artery, compared with venous occlusion plethysmography of the calf. *Pflugers Arch,* **382,** 241-248.

MACDONALD M.J., TSCHAKOVSKY M.E., AND HUGHSON R.L.. (1998).[In Press] Alveolar oxygen uptake and femoral artery blood flow dynamics in upright and supine exercise in humans. *J Appl Physiol*

MAGDER, S. (1990). Vascular mechanics of venous drainage in dog hindlimbs. *Am J Physiol,* **259,** H1789-H1795.

MAGDER, S. (1990). Starling resistor versus compliance: which explains the zero-flow pressure of a dynamic arterial pressure-flow relation? *Circ Res,* **67,** 209-220.

MAGDER, S. (1995). Venous mechanics of contracting gastrocnemius muscle and the muscle pump theory. *J Appl Physiol,* **79,** 1930-1935.

MANCIA, G. AND MARK, A.L. (1983). Arterial baroreflexes in humans. *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow.* chapt.20,(pp. 755-793). Bethesda, MD: Am. Physiol. Soc.

MARSHALL, J.M., AND TANDON, H.C. (1984). Direct observations of muscle arterioles and venules following contraction of skeletal muscle fibres in the rat. *J Physiol,* **350,** 447-459.

MCCLOSKEY, D.I., AND MITCHELL, J.H. (1972). Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol,* **224,** 173-186.

MELLANDER, S., AND JOHANSSON, B. (1968). Control of resistance, exchange, and capacitance vessels in the peripheral circulation. *Pharmacol Rev,* **20,** 117-196.

MICCO, A.J. CW and Pulse Doppler Diagnostic System. US. 4819652. A61B 10/00.

MITTELSTADT, S.W., BELL, L.B., O'HAGAN, K.P., AND CLIFFORD, P.S. (1994). Muscle chemoreflex alters vascular conductance in nonischemic exercising skeletal muscle. *J Appl Physiol,* **77,** 2761-2766.

MITTELSTADT, S.W., BELL, L.B., O'HAGAN, K.P., SULENTIC, J.E., AND CLIFFORD, P.S. (1996). Muscle chemoreflex causes renal vascular constriction. *Am J Physiol,* **270,** H951-H956.

MOHAN, J., AND MARSHALL, J.M. (1994). Responses evoked in the forearm vasculature on normal human subjects on repetition of mild, indirect cooling. *Clin Auto Res,* **4,** 29-34.

MOHRMAN, D.E., AND SPARKS, H.V. (1974). Myogenic hyperemia following brief tetanus of canine skeletal muscle. *Am J Physiol,* **227,** 531-535.

NAAMANI, R., HUSSAIN, S.N.A., AND MAGDER, S. (1995). The mechanical effect of contractions on blood flow to the muscle. *Eur J Appl Physiol,* **71,** 102-112.

NIELSEN, H.V. (1982). Effect of vein pump activation upon muscle blood flow and venous pressure in the human leg. *Acta Physiol Scand,* **114,** 481-485.

NIELSEN, H.V. (1983). Arterial pressure-blood flow relations during limb elevation in man. *Acta Physiol Scand,* **118,** 405-413.

NIELSEN, H.V. (1991). Transmural pressures and tissue perfusion in man. *Acta Physiol Scand,* **143(Suppl. 603),** 85-92.

NIELSEN, H.V., STABERG, B., NIELSEN, K., AND SEJRSEN, P. (1988). Effects of dynamic leg exercise on subcutaneous blood flow rate in the lower limb of man. *Acta Physiol Scand,* **134,** 513-518.

O'HAGAN, K.P., CASEY, S.M., AND CLIFFORD, P.S. (1997). Muscle chemoreflex increases renal sympathetic nerve activity during exercise. *J Appl Physiol,* **82,** 1818-1825.

O'LEARY, D.S., ROBINSON, E.D., AND BUTLER, J.L. (1997). Is active skeletal muscle functionally vasoconstricted during dynamic exercise in conscious dogs? *Am J Physiol,* **272,** R386-R391.

O'LEARY, D.S., AND SHERIFF, D.D. (1995). Is the muscle metaboreflex important in control of blood flow to ischemic active skeletal muscle in dogs? *Am J Physiol,* **268,** H980-H986.

OHYANAGI, M., FABER, J.E., AND NISHIGAKI, K. (1991). Differential activation of alpha1- and alpha2-adrenoreceptors on microvascular smooth muscle during sympathetic nerve stimulation. *Circ Res,* **68,** 232-244.

PERMUTT, S., AND RILEY, R.L. (1963). Hemodynamics of collapsible vessels with tone: the vascular waterfall. *J Appl Physiol,* **18,** 924-932.

PETERSEN, J.L., AND SINDRUP, J.H. (1990). Cutaneous blood flow rates during orthostatic

manoeuvres measured by laser Doppler flowmetry. *Acta Derm Venereol (Stockh),* **70,** 144-147.

PETERSON, D.F., ARMSTRONG, R.B., AND LAUGHLIN, M.H. (1988). Sympathetic neural influences on muscle blood flow in rats during submaximal exercise. *J Appl Physiol,* **65,** 434-440.

PHILLIPS, F.A.J., BRIND, S.H., AND LEVY, M.N. (1955). The immediate influence of increased venous pressure upon resistance to flow in the dog's hind leg. *Circ Res,* **3,** 357-362.

POLLACK, A.A., AND WOOD, E.H. (1949). Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. *J Appl Physiol,* **1,** 649-662.

RADDA, G.K. (1996). Control of energy metabolism during muscle contraction. *Diabetes,* **45,** S88-S92.

RADEGRAN, G. (1997). Ultrasound Doppler estimates of femoral artery blood flow during dynamic knee extensor exercise in humans. *J Appl Physiol,* **83,** 1383-1388.

RADEGRAN, G., AND SALTIN, B. (1998). Muscle blood flow at onset of dynamic exercise in humans. *Am J Physiol,* **274,** H314-H322.

READ, R.C., KUIDA, H., AND JOHNSON, J.A. (1958). Venous pressure and total peripheral resistance in the dog. *Am J Physiol,* **192,** 609-612.

REMENSNYDER, J.P., MITCHELL, J.H., AND SARNOFF, S.J. (1962). Functional sympatholysis during muscular activity. *Circ Res,* **11,** 370-380.

RICHARDSON, R.S., KENNEDY, B., KNIGHT, D.R., AND WAGNER, P.D. (1995). High muscle blood flows are not attenuated by recruitment of additional muscle mass. *Am J Physiol,* **269,** H1545-H1552.

RICHTER, E.A., KIENS, B., HARGREAVES, M., AND KJAER, M. (1992). Effect of arm-cranking on leg blood flow and noradrenaline spillover during leg exercise in man. *Acta*

Physiol Scand, **144,** 9-14.

ROBERGS, R.A., ICENOGLE, M.V., HUDSON, T.L., AND GREENE, E.R. (1997). Temporal inhomogeneity in brachial artery blood flow during forearm exercise. *Med Sci Sports Exerc,* **29,** 1021-1027.

ROCA, J., HOGAN, M.C., STORY, D., BEBOUT, D.E., HAAB, P., GONZALEZ, R., UENO, O., AND WAGNER, P.D. (1989). Evidence for tissue diffusion limitation of VO_2 max in normal humans. *J Appl Physiol,* **67,** 291-299.

RORIE, D.K., RUSCH, N.J., SHEPHERD, J.T., VANHOUTTE, P.M., AND TYCE, G.M. (1981). Prejunctional inhibition of norepinephrine release caused by acetylcholine in the human saphenous vein. *Circ Res,* **49,** 337-341.

ROTHE, C.F. (1983). Venous system: physiology of the capacitance vessels. In J.T. Shepherd, F.M. Abboud & S.R. Geiger (Eds.), *Handbook of Physiology. Section 2: The Cardiovascular System.* chapt.13,(pp. 397-452). Bethesda, MD: American Physiological Society.

ROWELL, L.B. (1988). Muscle blood flow in humans: how high can it go? *Med Sci Sports Exerc,* **20,** S97-S103.

ROWELL, L.B. (1993). *Human Cardiovascular Control.* New York: Oxford University Press.

ROWELL, L.B. (1997). Neural control of muscle blood flow: importance during dynamic exercise. *Clin Exp Pharmacol Physiol,* **24,** 117-125.

ROWELL, L.B., AND O'LEARY, D.S. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J Appl Physiol,* **69,** 407-418.

ROWELL, L.B., SALTIN, B., KIENS, B., AND CHRISTENSEN, N.J. (1986). Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? *Am J Physiol,*

251, H1038-H1034.

ROWELL, L.B., SAVAGE, M.V., CHAMBERS, J., AND BLACKMON, J.R. (1991). Cardiovascular responses to graded reductions in leg perfusion in exercising humans. *Am J Physiol,* **261,** H1545-H1553.

ROWLANDS, D.J., AND DONALD, D.E. (1968). Sympathetic vasoconstrictive responses during exercise- or drug-induced vasodilatation. *Circ Res,* **23,** 45-60.

RYGAARD, K., MOLLER, M., AND HENRIKSEN, O. (1991). Presence of nerve fiber collaterals from the sympathetic arteriolar plexus to the concomitant venules in dog skeletal muscle. *Acta Physiol Scand,* **143(Suppl. 603),** 115-118.

SAITO, M., KAGAYA, A., OCITA, F., AND SHINOHARA, M. (1992). Changes in muscle sympathetic nerve activity and calf blood flow during combined leg and forearm exercise. *Acta Physiol Scand,* **146,** 449-456.

SAITO, M., MANO, T., AND IWASE, S. (1990). Changes in muscle sympathetic nerve activity and calf blood flow during static handgrip exercise. *Eur J Appl Physiol,* **60,** 277-281.

SALTIN, B. (1988). Capacity of blood flow delivery to exercising skeletal muscle in humans. *Am J Cardiol,* **62,** 30E-35E.

SALTIN, B. (1989). Norepinephrine spillover from skeletal muscle during exercise in humans: role of muscle mass. *Am J Physiol,* **257,** H1812-H1818.

SAUPE, K.W., SMITH, C.A., HENDERSON, K.S., AND DEMPSEY, J.A. (1995). Diastolic time: an important determinant of regional arterial blood flow. *Am J Physiol,* **269,** H973-H979.

SAVARD, G., RICHTER, E.A., STRANGE, S., KIENS, B., CHRISTENSEN, N.J., AND SEALS, D.R. (1989). Sympathetic neural discharge and vascular resistance during exercise in

humans. *J Appl Physiol,* **66,** 2472-2478.

SECHER, N.H., CLAUSEN, J.P., NOER, I., AND TRAP-JENSEN, J. (1977). Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiol Scand,* **100,** 288-297.

SHEPHERD, J.T. (1983). Circulation to skeletal muscle. In J.T. Shepherd, F.M. Abboud & S.R. Geiger (Eds.), *Handbook of Physiology. The Cardiovascular System: Peripheral Circulation and Organ Blood Flow.* chapt.11, Bethesda, MD. American Physiological Society.

SHEPHERD, J.T., AND VANHOUTTE, P.M. (1981). Local modulation of adrenergic neurotransmission. *Circulation,* **64,** 655-666.

SHERIFF, D.D., ROWELL, L.B., AND SCHER, A.M. (1993). Is rapid rise in vascular conductance at onset of dynamic exercise due to muscle pump? *Am J Physiol,* **265,** H1227-H1234.

SHERIFF, D.D., AND VAN BIBBER, R. (1998). Flow-generating capability of the isolated skeletal muscle pump. *Am J Physiol,* **274,** H1502-H1508.

SHERIFF, D.D., WYSS, C.R., ROWELL, L.B., AND SCHER, A.M. (1987). Does inadequate oxygen delivery trigger pressor response to muscle hypoperfusion during exercise? *Am J Physiol,* **253,** H1199-H1207.

SHOEMAKER, J.K., HALLIWILL, J.R., HUGHSON, R.L., AND JOYNER, M.J. (1997). Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery. *Am J Physiol,* **273,** H2388-H2395.

SHOEMAKER, J.K., HODGE, L., AND HUGHSON, R.L. (1994). Cardiorespiratory kinetics and femoral artery blood velocity during dynamic knee extension exercise. *J Appl Physiol,* 77,

2625-2632.

SHOEMAKER, J.K., NAYLOR, H.L., POZEG, Z.I., AND HUGHSON, R.L. (1996). Failure of prostaglandins to modulate the time course of blood flow during dynamic forearm exercise in humans. *J Appl Physiol,* **81,** 1516-1521.

SHOEMAKER, J.K., PANDEY, P., HERR, M.D., SILBER, D.H., YANG, Q.X., SMITH, M., GRAY, K., AND SINOWAY, L.I. (1997). Augmented sympathetic tone alters muscle metabolism during exercise and recovery: lack of metabolic evidence for functional sympatholysis. *J Appl Physiol,* **82,** 1932-1938.

SHOEMAKER, J.K., PHILLIPS, S.M., GREEN, H.J., AND HUGHSON, R.L. (1996). Faster femoral artery blood velocity kinetics at the onset of exercise following short-term training. *Cardiovasc Res,* **31,** 278-286.

SHOEMAKER, J.K., POZEG, Z.I., AND HUGHSON, R.L. (1996). Forearm blood flow by Doppler ultrasound during rest and exercise: tests of day-to-day repeatability. *Med Sci Sports Exerc,* **28,** 1144-1149.

SHOEMAKER, J.K., TSCHAKOVSKY, M.E., AND HUGHSON, R.L. (1998). Vasodilation contributes to the rapid hyperemia with rhythmic contractions in humans. *Can J Physiol Pharmacol,* **76,** 1-10.

SHRIER, I., BARATZ, A., AND MAGDER, S. (1997). Effects of adenosine on pressure-flow relationships in an in vitro model of compartment syndrome. *J Appl Physiol,* **82,** 755-759.

SHRIER, I., AND MAGDER, S. (1993). Response of arterial resistance and critical closing pressure to changes in perfusion pressure in canine hindlimb. *Am J Physiol,* **265,** H1939-H1945.

SHRIER, I., AND MAGDER, S. (1995). Maximal vasodilation does not eliminate the vascular

waterfall in the canine hindlimb. *J Appl Physiol,* **79,** 1531-1539.

SINOWAY, L., AND PROPHET, S. (1990). Skeletal muscle metaboreceptor stimulation opposes peak metabolic vasodilation in humans. *Circ Res,* **66,** 1576-1584.

SINOWAY, L., PROPHET, S., GORMAN, I., MOSHER, T., SHENBERGER, J., DOLECKI, M., BRIGGS, R., AND ZELIS, R. (1989). Muscle acidosis during static exercise is associated with calf vasoconstriction. *J Appl Physiol,* **66,** 429-436.

SINOWAY, L.I., SMITH, M.B., ENDERS, B., LEUENBERGER, U., DZWONCZYK, T., GRAY, K., WHISTLER, S., AND MOORE, R.L. (1994). Role of diprotonated phosphate in evoking muscle reflex responses in cats and humans. *Am J Physiol,* **267,** H770-H778.

SKAGEN, K. (1982). Contribution of local blood flow regulation mechanisms during head-up tilt in human subcutaneous tissue. *Acta Physiol Scand,* **116,** 331-334.

SPAAN, J. (1985). Coronary diastolic pressure-flow relation and zero flow pressure explained on the basis of intramyocardial compliance. *Circ Res,* **56,** 293-309.

STEGALL, H.F. (1966). Muscle pumping in the dependent leg. *Circ Res,* **19,** 180-190.

STICK, C., JAEGER, H., AND WITZLEB, E. (1992). Measurements of volume changes and venous pressure in the human lower leg during walking and running. *J Appl Physiol,* **72,** 2063-2068.

STRANDELL, T., AND SHEPHERD, J.T. (1967). The effect in humans of increased sympathetic activity on the blood flow to active muscles. *Acta Med Scand,* **472,** 146-167.

SUNDLOF, G., AND WALLIN, G. (1978). Effect of lower body negative pressure on human muscle nerve sympathetic activity. *J Physiol,* **278,** 525-532.

TERJUNG, R.A., AND MACKIE ENGBRETSON, B. (1988). Blood flow to different rat

skeletal muscle fiber type sections during isometric contractions in situ. *Med Sci Sports Exerc,* **20(5)Suppl.** S124-S130.

THOMAS, G.D., HANSEN, J., AND VICTOR, R.G. (1994). Inhibition of alpha2-adrenergic vasoconstriction during contraction of glycolytic, not oxidative, rat hindlimb muscle. *Am J Physiol,* **266,** H920-H929.

THOMPSON, L.P., AND MOHRMAN, D.E. (1983). Blood flow and oxygen consumption in skeletal muscle during sympathetic stimulation. *Am J Physiol,* **245,** H66-H71.

THORSSON, O., LILJA, B., AHLGREN, L., HEMDAL, B., AND WESTLIN, N. (1985). The effect of local cold application on intramuscular blood flow at rest and after running. *Med Sci Sports Exerc,* **17,** 710-713.

TRIPATHI, A., MACK, G., AND NADEL, E.R. (1989). Peripheral vascular reflexes elicited during lower body negative pressure. *Aviat Space Environ Med,* **60,** 1187-1193.

TRIPATHI, A., AND NADEL, E.R. (1986). Forearm skin and muscle vasoconstriction during lower body negative pressure. *J Appl Physiol,* **60,** 1535-1541.

TSCHAKOVSKY, M.E., SHOEMAKER, J.K., AND HUGHSON, R.L. (1995). Beat-by-beat forearm blood flow with Doppler ultrasound and strain-gauge plethysmography. *J Appl Physiol,* **79,** 713-719.

TSCHAKOVSKY, M.E., SHOEMAKER, J.K., AND HUGHSON, R.L. (1996). Vasodilation and muscle pump contribution to immediate exercise hyperemia. *Am J Physiol,* **271,** H1697-H1701.

TYBERG, J.V. AND BAKER, S.E. (1993). Venous capacitance changes in congestive heart failure and exercise. *Veins: Their functional role in the circulation.* (pp. 49-60). Tokyo: Springer-Verlag.

VAN LEEUWEN, B.E., BARENDSEN, G.J., LUBBERS, J., AND DE PATER, L. (1992). Calf blood flow and posture: Doppler ultrasound measurements during and after exercise. *J Appl Physiol,* **72,** 1675-1680.

VAN LEEUWEN, B.E., LUBBERS, J., BARENDSEN, G.J., AND DE PATER, L. (1992). Calf blood flow and posture: Doppler ultrasound calibrated by plethysmography. *J Appl Physiol,* **72,** 1668-1674.

VATNER, S.F., FRANKLIN, D., VAN CITTERS, R.L., AND BRAUNWALD, E. (1970). Effects of carotid sinus nerve stimulation on blood flow distribution in conscious dogs at rest and during exercise. *Circ Res,* **27,** 495-503.

VERHAEGHE, R.H., VANHOUTTE, P.M., AND SHEPHERD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circ Res,* **40,** 208-215.

VICTOR, R.G., BERTOCCI, L.A., PRYOR, S.L., AND NUNNALLY, R.L. (1988). Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *J Clin Invest,* **82,** 1301-1305.

VISSING, S.F., SCHERRER, U., AND VICTOR, R.G. (1994). Increase of sympathetic discharge to skeletal muscle but not to skin during mild lower body negative pressure in humans. *J Physiol (Lond),* **481,** 233-241.

VISSING, S.F., SECHER, N.H., AND VICTOR, R.G. (1997). Mechanisms of cutaneous vasoconstriction during upright posture. *Acta Physiol Scand,* **159,** 131-138.

WALKER, R.L., MACKAY, I.F.S., AND VAN LOON, P. (1967). Vascular responses to venous congestion. *J Appl Physiol,* **22,** 889-899.

WALLOE, L., AND WESCHE, J. (1987). Time course and magnitude of blood flow changes in the human quadriceps muscles during and following rhythmic exercise. *J Physiol,* **405,** 257-273.

WHITNEY, R.J. (1953). The measurement of volume changes in human limbs. *J Physiol,* **121,** 1-27.

WILLIAMS, C.A., MUDD, J.G., AND LIND, A.R. (1978). The forearm blood flow during intermittent hand-grip isometric exercise. *Circ Res,* **48,** I-110-I-117.

WILLIAMS, C.A., MUDD, J.G., AND LIND, A.R. (1985). Sympathetic control of the forearm blood flow in man during brief isometric contractions. *Eur J Appl Physiol,* **54,** 156-162.

WYSS, C.R., ARDELL, J.L., SCHER, A.M., AND ROWELL, L.B. (1983). Cardiovascular responses to graded reductions in hindlimb perfusion in exercising dogs. *Am J Physiol,* **245,** H481-H486.