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PERIPHERAL VS CENTRAL CONTROL OF CARDIAC OUTPUT

by

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ABSTRACT

Neural, humoral and mechanical factors affecting cardiac function and the peripheral vasculature were examined to assess the relative importance of peripheral vs cardiac factors in the control of cardiac output during exercise. First, I examined neural vs local regulation of humoral vasoconstrictors endothelin-1 (ET) and NPY, in anesthetized dogs, and showed that plasma ET levels, in contrast to NPY, increase in response to systemic hypotension but not carotid sinus baroreceptor activation. This did not occur with intact vagi suggesting an interaction of neural and humoral factors. In the next two studies I separated peripheral vascular and cardiac neural influences on cardiac output (CO) and right atrial pressure (Pra) responses during graded exercise by comparing cardiac denervated heart transplant patients (HT) with normally innervated subjects. The increase in Pra was higher in HT than normals but stabilized as the CO increased at peak effort. Stimulation of the heart with dobutamine in 2 patients did not increase exercise capacity, suggesting that peripheral not cardiac factors limit exercise in HT patients. The rise in central venous pressure at exercise onset was similar in both groups which demonstrates the importance of the mechanical effect of muscle contraction vs reflex changes in mobilizing blood from the peripheral vasculature at exercise onset. In the fourth study I assessed whether large changes in human body mass, induced by isolated gastric bypass

surgery, would affect the heart rate (HR)/oxygen consumption (VO2) relationship during exercise. Peak absolute $\dot{V}O2$ was significantly lower in the previously obese group vs obese and control groups despite similar normalized 24-hour energy expenditure. HR was higher in the previously obese at a given submaximal $\dot{V}O2$ due to the higher relative $\dot{V}O2$, suggesting a significant loss of muscle mass and supporting the idea that HR is a function of the relative $\dot{V}O2$. In the fifth study I assessed the influence of mechanical vs reflex effects of muscle contractions on venous mechanics during electrically stimulated dynamic exercise in anesthetized dogs. Increases in HR, CO and blood pressure during stimulation were reversed when thin-fibre muscle afferents were blocked. Mean circulatory filling pressure increased in both conditions and Pra tended to decrease during stimulation but not during nerve block. This suggests that the pressure gradient for venous return is increased during mild exercise as a result of reflex effects mediated by muscle afferents with a possible small mechanical effect of muscle contractions. Appropriate increases in HR and CO during dynamic exercise in this model, rely on neural feedback from exercising muscle.

RÉSUMÉ

Les facteurs nerveux, humoraux et mécaniques qui modulent les fonctions cardiaques et le système vasculaire périphérique ont été examinés pour évaluer l'importance relative des facteurs cardiaques par rapport aux facteurs périphériques dans le contrôle du débit cardiaque pendant l'effort. J'ai comparé en premier lieu la régulation locale et la régulation nerveuse des vasoconstricteurs humoraux endothéline-1 (ET) et NPY sur des chiens anesthésiés et démontré que les concentrations plasmatiques de ET, augmentaient en cas d'hypotension systémique mais pas en cas d'activation du barorécepteur du sinus carotidien, ce qui n'est pas le cas des concentrations plasmatiques de NPY. Ce phénomène ne s'observe pas lorsque les nerfs pneumogastriques sont intacts ce qui donne à penser qu'il y a interaction des facteurs nerveux et humoraux. Dans le cadre de deux autres études, j'ai séparé les influences vasculaires périphériques et les influences nerveuses cardiaques sur les réponses du débit cardiaque (DC) et de la pression auriculaire droite (Par) dans le cadre de séances d'exercice graduel, en comparant des transplantés cardiaques (TC) dont le coeur avait subi une énervation à des sujets normalement innervés. L'augmentation de la Par a été supérieure chez les transplantés que chez les sujets normaux mais s'est stabilisée à mesure que le DC augmentait au maximum de l'effort. L'excitation du coeur avec de la dobutamine chez 2 patients n'a pas accru leur capacité d'effort, ce qui donne

à penser que ce ne sont pas les facteurs cardiaques qui limitent la capacité d'effort des patients ayant subi une transplantation mais plutôt les facteurs périphériques. L'augmentation de la pression veineuse centrale au début de l'effort a été identique dans les deux groupes ce qui prouve l'importance de l'effet mécanique de la contraction musculaire par rapport aux changements de réflexe dans la mobilisation du sang dans le système vasculaire périphérique au début de l'effort. Dans le cadre d'une quatrième étude, j'ai cherché à savoir si des changements importants dans la masse corporelle humaine, induits par courts-circuits gastriques isolés, affectaient le rapport entre le rythme cardiaque (RC) et la consommation d'oxygène ($\dot{V}O2$) pendant l'effort. Le maximum absolu de VO2 a été significativement inférieur dans le groupe formé d'anciens obèses, par rapport au groupe d'obèses et au groupe témoin, malgré une dépense énergétique normalisée identique. Le rythme cardiaque était supérieur dans le groupe des anciens obèses avec une $\dot{V}O2$ sous maximale attribuable à une $\dot{V}O2$ relative supérieure, traduisant une diminution significative de la masse musculaire et confirmant l'hypothèse selon laquelle le rythme cardiaque dépend de la $\dot{V}O2$ relative. Dans la cinquième étude, j'ai évalué l'influence des effets mécaniques par rapport aux effets réflexes de la contraction musculaire sur la mécanique veineuse, dans le cadre d'efforts dynamiques stimulés électriquement, sur des chiens anesthésiés. L'augmentation du

rythme cardiaque, du DC et de la tension artérielle pendant l'excitation ont été inversées lorsque les muscles à fibres minces afférents ont été bloqués. La pression de remplissage circulatoire moyenne a augmenté et la Par a eu tendance à diminuer pendant l'excitation mais pas pendant le blocage nerveux. Cela semble indiquer que le gradient de pression du retour veineux augmente en cas d'efforts modérés à cause des effets réflexes modulés par les muscles afférents avec une petite effets des mécaniques de la contraction musculaire. L'augmentation adéquate du rythme cardiaque et du DC en phase d'effort dynamique dans ce modèle dépend de la rétroaction nerveuse du muscle qui fournit l'effort.

For Mom and Dad

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I would like to sincerely thank my supervisor Dr. S. Magder from whom I have learned so much. His love of science and enthusiasm for research and teaching are an enormous benefit to his students. I consider myself fortunate to be one of them. I now know that if you are to climb Mount Everest, you must have a good reason.

I appreciate the technical assistance I received from Steve Nuara, without whom my animal experiments would not be possible. I thank Joan Longo and Roberta Carin for their help with papers and grants over the years. I am grateful for the financial assistance from the Research Institute of the Royal Victoria Hospital and the American College of Sports Medicine Foundation.

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PREFACE

This thesis is divided into seven chapters. The introduction in Chapter 1 provides background information and a review of the literature pertinent to the thesis. In chapters 2,3,4,5 and 6, I have opted to include original papers submitted for publication as per "Guidelines Concerning Thesis Preparation" of McGill University. The study described in chapter 2 has been accepted for publication by the Canadian Journal of Physiology and Pharmacology. The studies outlined in chapters 3 and 4 are under review at Journal of Applied Physiology and the Canadian Journal of Physiology and Pharmacology, and those in chapters 5 and 6 are being submitted to the Annals of Internal Medicine and the Journal of Applied Physiology respectively. The conclusions of the thesis are discussed in chapter 7 and are followed by Claims of Originality.

The experiments in chapter 2 were performed with the assistance of Dr.F.Erice, a fellow in the Critical Care Division, and Dr.D.Stewart of the Cardiology Department of the Royal Victoria Hospital and their names appear as co-authors on the paper. The experiment in chapter 3 was conducted with the assistance of Dr.R.D.Levy and Ann Tully of the Respiratory Division and Dr.D.Fitchett of the Cardiology Department of the Royal Victoria Hospital and their names appear as co-authors on the paper. B.Rhode and Dr.L.D.MacLean of the Department of

Surgery assisted in organizing the study in chapter 5 and their names appear as co-authors on the paper. My supervisor, Dr.S.Magder is the senior author on all manuscripts published or under review.

I have used the American Physiological Society guidelines for the format of chapters 1-6 and in the units of measurements. The corresponding SI units are listed in Appendix I. Appendix II is a list of the abbreviations used in the thesis.

CHAPTER 1

Introduction

A. CENTRAL CARDIAC AND PERIPHERAL VASCULAR FACTORS AFFECTING CARDIAC OUTPUT AND VENOUS RETURN

The function of the cardiovascular system is to transport oxygen and nutrients from one part of the body to another and exchange these in organs and tissues for metabolic waste products which can then be removed. The circulatory system is made up of a central pump, the heart, an oxygenating unit, the lungs, and a peripheral vasculature which includes branching arterial, capillary and venous vessels. Approximately 75% of the total blood volume is contained in the venous system, which acts as a large reservoir from which blood drains back to the heart.

Cardiac output is the amount of blood pumped out of the heart in a given time and in humans at rest is approximately 5 - 6 litres per minute. In the steady state, this must be equal to the amount of blood returning to the right atrium of the heart, which is the venous return. Cardiac output increases dramatically during dynamic muscular exercise and maximal values as high as 35-40 litres per minute have been reported in endurance athletes (34). The mechanisms by which this occurs must involve changes in both heart and peripheral circuit parameters since they are mechanically coupled (48). However, the traditional view of the circulation is one where the properties of the heart alone dictate changes in cardiac output (125,147). This view is still widely held today and is based on a model of the circulation where the heart pumps

blood into a rigid tube which returns the blood to the intake of the pump. However, the heart has a filling phase as well as an emptying phase and therefore requires a non-rigid circuit which can provide changes in volume i.e. compliance as well as resistance. In fact the circulation is a closed circular system of non-rigid tubular structures with a defined blood volume. The veins are 18-30 times more compliant than the arteries (51) and hold the bulk of blood volume, providing a reservoir from which blood returns to the heart (157). Therefore, venous circuit parameters largely determine the flow of blood back to the heart which must equal the cardiac output in the steady state.

The central cardiac and peripheral vascular factors which affect venous return and therefore cardiac output will be discussed in the next sections.

Central Cardiac Factors

The heart has the intrinsic ability to increase its output when the blood flow it receives increases. This is the Frank-Starling law of the heart and allows the heart to rapidly adjust to sudden changes in venous return and equate the output of the right and left sides of the heart according to the length/tension relationship of the ventricles. The greater the length of the myocardial fibres or diastolic volume, the greater is the tension and degree of shortening generated by the muscle contraction. The increase in the volume of blood ejected during contraction (stroke volume) is directly related

to the increase in end-diastolic volume. Therefore, the initial stretch or preload of the cardiac muscle fibre determines its isometric tension and influences cardiac function (144). Other factors are the force against which the heart must pump, or afterload and the frequency of contraction, or heart rate. The force of contraction itself, or contractility also affects cardiac performance. These latter 3 factors are influenced by neural and humoral factors in the intact circulation, and a change in one factor influences the others. This led to a controversy in the early literature as to whether the Starling Law applied to the intact human since independent control of these interdependent cardiac factors in the intact circulation was more difficult than in Starling's isolated heart-lung preparation (133).

The effects of preload, afterload, heart rate and contractility on the function of the heart as a pump have been studied by using ventricular or cardiac function curves (49,106,133).

The relationship between cardiac output (or stroke volume at a constant heart rate) and ventricular filling pressure, known as the Starling curve, was proposed by Starling based on experiments using an isolated heart-lung preparation and applied to both right and left sides of the heart (106). He found an early steep increase in stroke volume with increasing filling pressure which he explained on the basis of the length/tension relationship of the cardiac muscle. This

property becomes limited when heart filling is maximal, i.e. the myocardial fibres cannot be stretched further, and the filling pressure of the heart then increases without a further increase in the volume ejected. This later plateau in the curve was referred to by Starling as the "maximal output" of a given heart. Patterson and Starling also showed that this "maximal output" was higher the lower the arterial resistance (or afterload) and the higher the heart rate. Sarnoff (133) later expanded on this idea and demonstrated that there were a family of Starling or ventricular function curves, in the intact circulation which explained variations in measurements under different physiological conditions. Neural or humoral-induced increases in heart rate, contractility or decreases in afterload would act to shift the curve to the left and increase cardiac function, i.e. a greater volume would be ejected for a given filling pressure. The reverse would shift the curve to the right, decreasing cardiac function, where a lower volume would be ejected at a given filling pressure (Figure 1.1).

However, the degree to which increases in heart rate and contractility can increase cardiac output is limited since each can only affect the return of blood to the right atrium by lowering right atrial pressure, the downstream pressure of the systemic circulation. This occurs as a result of the low pressure in the right atrium under normal conditions and an inflow limitation which develops when right atrial pressure

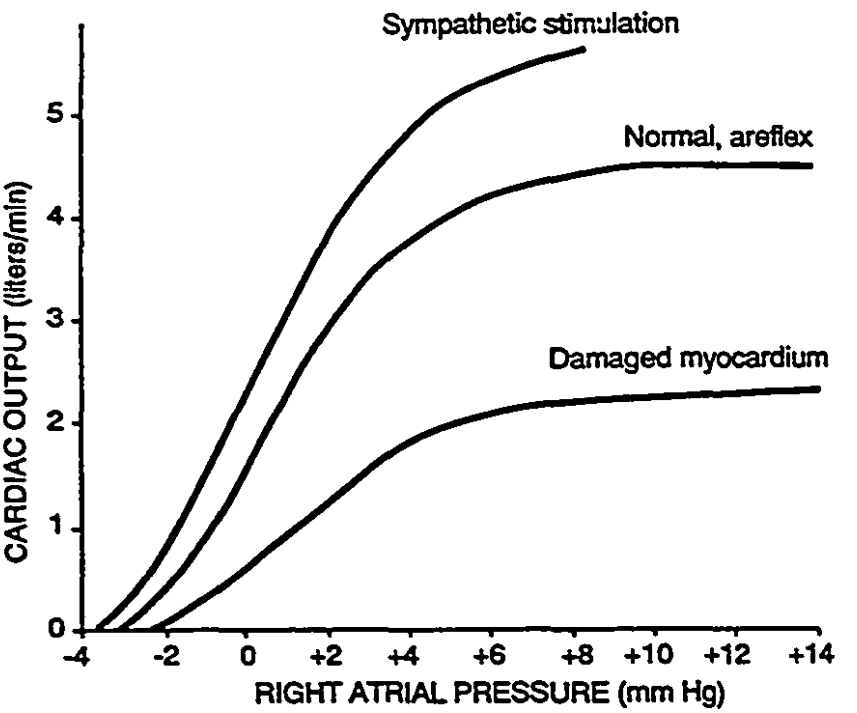

Figure 1.1. Cardiac response curves under normal or areflexic conditions, increased sympathetic stimulation (*shift to left*) and myocardial damage (*shift to right*). Reprinted from Guyton et.al. Physiol.Rev.35:123-129, 1955 with permission.

falls below atmospheric pressure (157). When right atrial pressure falls below atmospheric pressure, the great veins leading back to the heart demonstrate vascular waterfall behaviour such that flow in the venae cavae becomes constant and independent of further reductions in right atrial or downstream pressure (50,108). This collapse causes the pressure in the veins, where they first enter the chest cavity, to remain at approximately zero mmHg irrespective of how negative the pressure becomes in the right atrium (50). The phenomenon has been paralleled with maximal expiratory flow limitation in the lung (108). This potential limitation of cardiac factors to increase cardiac output was demonstrated effectively in humans both with and without complete heart block, by artificially pacing the heart (7,118). The authors sought to assess the contribution of heart rate and contractility in the 5 to 8-fold increase in cardiac output reported during dynamic exercise (72). Both studies found that varying heart rate by pacing at rest between approximately 70 and 120 beats per minute (bt/min) resulted in a corresponding decrease in stroke volume such that cardiac output was unchanged. A similar effect was noted during exercise when heart rate was prevented from changing. A slight decrease in cardiac output was found when rates were increased to 150 bt/min or above. This occurred because of the shortened diastolic filling time at higher heart rates which resulted in a decreased diastolic volume since venous return remained

constant. Any further increase in cardiac output must result from an increase in venous return. The authors recognized the importance of a reduced diastolic filling time when heart rate was increased in patients with complete heart block and added that another factor could be the lack of coordination between atrial and ventricular systole in these patients. Both studies suggested that cardiac output is regulated through mechanisms other than heart rate at rest and this may be a function of active regulation of blood flow to peripheral metabolic demands during exercise (7,118).

Guyton integrated the analysis of the early studies and measured cardiac function curves, which he referred to as cardiac response curves, in the intact anesthetized dog by a technique of administering massive rapid blood transfusions and making measurements of cardiac output and right atrial pressure before complete compensatory readjustments could occur (54). He demonstrated that the factors affecting cardiac response, preload, afterload, contractility and heart rate, can be influenced on a beat to beat or steady state basis by other factors such as respiratory phase, degree of sympathetic stimulation, degree of oxygenation of the blood, myocardial damage and cardiac fatigue. However, under normal resting conditions with a constant degree of sympathetic stimulation, the cardiac response or function curve remains relatively constant from beat to beat. By defining the peripheral factors affecting venous return and expressing these graphically in

the form of a venous return curve which could be plotted on the same axes as the cardiac function curve, he identified the intersection point of the two curves as the actual steady state cardiac output or venous return and drew attention to the importance of the venous waterfall or collapse behaviour (49,50). In so doing, Guyton emphasized that changes in cardiac function are limited in the extent to which they can increase cardiac output, except when cardiac filling is maximal. Peripheral vascular factors which determine venous return are of major importance as the heart can only alter the flow of blood returning to it by altering the common variable of right atrial pressure through changes in pump function (49,50). These peripheral vascular factors and their role in the control of venous return and cardiac output are discussed in the next sections.

Peripheral Vascular Factors

As early as 1850, a model of the circulation which emphasized peripheral vascular control was proposed by Weber (172). In a simple model made from a loop of small intestine fitted with valves to simulate the heart and a sponge to represent the microcirculation, he was able to demonstrate that at zero flow the pressure in the loop was the same everywhere. This pressure was termed "hydrostatic mean pressure" and was found to be equal to the average pressure in the system during flow. Weber concluded that the pump in such a system cannot increase the mean pressure that is exerted on the walls of the tubular

system by the fluid within them. Therefore the role of the pump, representing the heart, is to bring about an unequal pressure distribution by reducing pressure in the veins by pumping fluid out of them and increasing pressure in the arteries by pumping fluid into them. The mean pressure in this model can only be increased by distension of the tubes by infusion of more fluid. This was the first recognition of what would later be termed "mean systemic pressure" or "mean circulatory filling pressure". Bayliss and Starling adopted this theory in order to study the mechanism responsible for peripheral edema during heart failure (6) and measured a mean systemic pressure of 10 mmHg when the heart was stopped by vagal stimulation and pressures allowed to equalize in the anesthetized dog. They recognized that during normal cardiac activity, the pressure fall on the venous side is not proportional to the rise on the arterial side due to the difference in compliance or distensibility as well as capacity of the two types of vessels. They reasoned that somewhere in the intact circulation there must be a point where the pressure is neither raised nor lowered and is therefore independent of cardiac activity. This point was most likely in the veins as evidenced by the lack of change in portal vein pressure when the heart was stopped (6,143). The mean systemic pressure could be raised by active vasoconstriction or an increase in blood volume or compression of the vessels from without (6,146) and was thought to be responsible for venous

congestion in heart failure, rather than the failing heart itself. This was corroborated in human studies performed decades later in 1940 by Starr. He measured mean systemic pressure in patients immediately after death and found that it correlated directly with the degree of congestion (145). Krogh also supported the idea of the importance of the venous vasculature in regulating venous return and thereby cardiac output (70). As a result of the enormous increases in blood flow seen during muscular exercise which could not be explained merely by arteriolar dilation in the muscle, he proposed that the portal or splanchnic system was an important regulator of the central venous pressure and therefore the supply of blood to the right heart (71). This region has a large capacitance and acts as a reservoir to supply blood to the central venous vessels. This would compensate for the muscular vasodilation seen during exercise, thereby maintaining mean arterial pressure. Krogh also identified two mechanisms which influence the pressure in the central vein. They were the general tone of the venous system and respiratory movements (71).

These early works implicated several factors in the peripheral vasculature through which venous return to the heart may be determined. They are: mean systemic pressure, which varies with the venous capacitance and compliance; venous tone or resistance; and distribution of blood flow between portal and other regions as a function of vasomotor

changes.

Guyton further defined these circuit factors mathematically by experiments in which he constructed venous return curves by replacing the heart with a pump. As pump rate was gradually decreased to zero and venous return ceased, right atrial pressure rose until it was equal to mean systemic pressure, which he referred to as mean circulatory filling pressure (MCFP). Therefore, the pressure gradient for venous return is the difference between MCFP (the upstream pressure) and right atrial pressure (the downstream pressure). These are the two major pressure factors which determine the quantity of blood returning to the heart from the peripheral circulatory system. By varying the total amount of blood in the circulatory system of the dog by hemorrhage and transfusion which decreased and increased MCFP respectively, Guyton was able to construct a family of parallel venous return curves under conditions of constant resistance. This demonstrated the large effect of vascular volume, or that portion of it which contributes to the pressure in the vessels (stressed vascular volume), on venous return and cardiac output (Figure 1.2) (49). When right atrial pressure was decreased below zero, venous return or cardiac output became constant due to the venous waterfall or collapse factor discussed previously. The effect of an increasing venous resistance on these curves was achieved by progressively occluding the veins entering the right atrium. While venous resistance is small compared with arterial

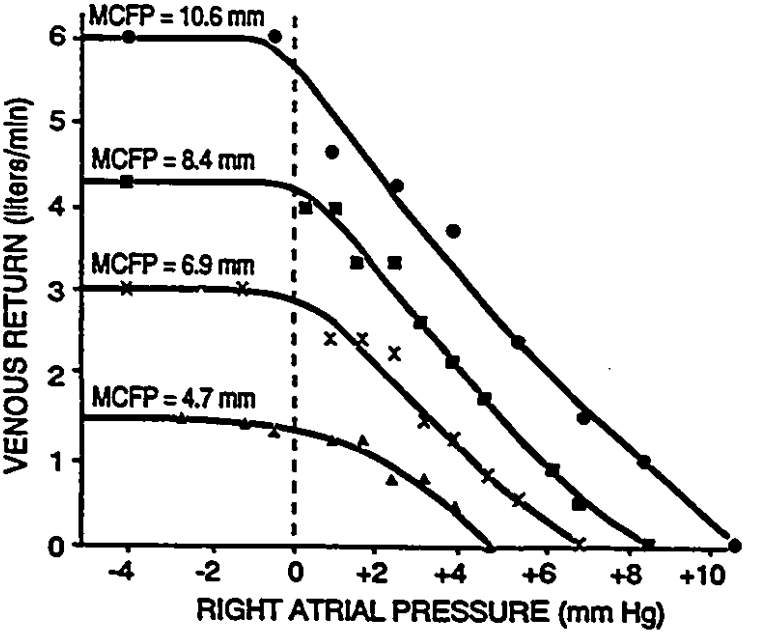

Figure 1.2. Venous Return Curves. See text for details. Reprinted from Guyton et.al. Physiol.Rev.35:123-129, 1955 with permission.

resistance, increasing the latter had only a slight effect on venous return, whereas increasing the resistance between the large blood reservoirs in the venules and the right atrium caused a dramatic decrease in venous return. Guyton expressed these relationships by the following formula:

$$V.R. = \frac{MCFP - Pra}{Rv}$$

Where: V.R. is venous return which must equal cardiac output in the steady state; MCFP is mean circulatory filling pressure; Pra is right atrial pressure; and Rv is resistance to venous return.

Caldini (10) expanded this relationship and incorporated Krogh's model (71) to describe two compartments in the circulation distinguished by slow and fast time constants of venous drainage, which reflect different compliances and resistances in parallel circuit compartments. Similar to Krogh, the splanchnic region was proposed to be the slow time constant region. Changes in the fractional distribution of blood flow between these two lumped regions could result in large changes in venous return. Caldini studied this with a right heart bypass technique which allowed independent control of blood flow and right atrial pressure and precise measurement of volume changes in the bypass reservoir. Since the compliance of the arterial system is minimal in comparison with the venous, this model assumes that all of the vascular compliance is in the small veins and venules.

While studying the effects of epinephrine on the peripheral

circulation and cardiac output, Caldini and associates demonstrated that step decreases in right atrial pressure at a constant flow resulted in relationships between volume and time that could only be accounted for by the sum of two exponentials of widely different time constants. Since venous parameters were largely unaffected, they concluded that epinephrine-induced increases in steady state venous return, at a constant right atrial pressure, resulted from the drug's effect on the distribution of blood flow within the parallel compartments. The slope of the venous return curve, the resistance to venous return, was markedly affected by the distribution of blood flow between the two compartments, which was largely determined by changes in arterial resistance. Guyton's equation for venous return was expanded as follows (10):

$$V.R. = (v - PraCt)/(FfTf + FsTs)$$

Where: V.R. is venous return; v is the stressed vascular volume; Pra is right atrial pressure; Ct is the total compliance; Ff and Fs are the fractional distribution of flow to the fast and slow time constant beds respectively; Tf and Ts are the time constants of the fast compartment and slow compartments of venous drainage respectively.

These time constants of venous drainage have been measured in subsequent studies by separating systemic venous return into splanchnic and extrasplanchnic flows and have confirmed

that the slow time constant bed was located in the splanchnic region (26,45,99). Measures of the time constant for the splanchnic region ranged from 17 to 24 seconds. They reported extrasplanchnic time constants in the range of 4-8 seconds. More recently, Magder (83) found that the time constant for venous drainage in the isolated dog hindlimb, measured both by changing inflow and venous pressure, was 4-5 seconds. A double occlusion technique was used to measure pressure in the compliant region and to calculate venous resistance.

The peripheral circuit factors discussed thus far are modifiable by various humoral, neural and mechanical factors. Epinephrine administration was found by Caldini to primarily alter regional blood flow distribution (10), while others found an effect on venous tone (53,99). This question was settled by Deschamps and Magder (26) who showed that low carotid sinus pressure resulted in a decrease in splanchnic venous resistance and capacitance thereby decreasing the splanchnic time constant and altering blood flow distribution, and high carotid pressure resulted in the opposite effect. These authors noted that the passive blood flow redistribution observed by Caldini in response to epinephrine infusion could be explained by carotid baroreceptor stimulation.

Mechanical factors such as skeletal muscle contraction in the hindlimb and the abdomen as shown by Guyton et.al. (52) can also instantaneously raise mean systemic pressure, thus increasing the gradient for venous return and translocating

blood centrally.

Determinants of Venous Return

The pressure gradient for venous return is determined by the difference between the mean systemic pressure and right atrial pressure. Mean systemic pressure is determined by stressed vascular volume (total blood volume minus the unstressed volume or capacitance) and total compliance. Right atrial pressure is determined both by changes in cardiac function and the peripheral vascular circuit, the actual value reflecting the relative changes in each i.e. the pressure at the intersection point of the cardiac response and venous return curves.

Venous compliance and resistance of each vascular bed determines the time constant for venous drainage. These time constants as well as the fractional flow to each region determines the resistance to venous return.

In summary, the five determinants of venous return are: stressed vascular volume, venous compliance, right atrial pressure, venous resistance and the distribution of blood flow between slow and fast time constant beds.

B. LIMITATIONS TO OXYGEN CONSUMPTION DURING DYNAMIC EXERCISE

Maximal cardiac output possible during dynamic exercise stress, whether in an animal or human, occurs at the maximal oxygen consumption of that individual. Many authors have

demonstrated that cardiac output increases in direct proportion to the increase in the absolute oxygen consumption of the whole body (17,33,36,39,89). In contrast, heart rate increases linearly with increases in the relative oxygen consumption, i.e. the percentage of the individual's maximum oxygen consumption (17). Therefore, heart rate is effort dependant whereas cardiac output is dependant on the actual oxygen cost of the work performed. Since oxygen consumption is dependant on cardiac output, much of the work related to what limits maximal oxygen consumption, in the last century, has focused on the limits of oxygen transport by the circulation, in particular, the ability of the heart to increase its output. However, as I have discussed previously, the increase in cardiac output is dependant on cardiac factors and circuit factors.

The convective transport of oxygen by the circulatory system can be described by the Fick equation: $\dot{V}O2 = \dot{Q} \cdot (CaO2 - C\bar{v}O2)$;

which states that the steady state oxygen consumption of the whole animal ($\dot{V}O2$) is the product of the cardiac output ($\dot{Q}$) multiplied by the difference in oxygen content of arterial ($CaO2$) and mixed venous ($C\bar{v}O2$) blood. In the previous section, I reviewed the factors influencing cardiac output. The arterial-mixed venous oxygen content difference is determined by: the oxygen carrying capacity of the blood, which depends on the hemoglobin concentration, the saturation of the

arterial blood, and the degree of oxygen extraction in the systemic circulation. The latter depends to some extent on the distribution of blood flow between metabolically active and relatively inactive regions.

In order to identify potential limiting factors in maximal oxygen consumption along the oxygen pathway from ambient air to utilization in the exercising muscle, I will first review this oxygen cascade.

Oxygen Transport Cascade

The "oxygen cascade" refers to the progressive decline in the partial pressure of oxygen (PO2) of air as it passes from the mouth to the alveoli of the lung, across the alveolar membrane to the red blood cells, through the arterial tree to tissue capillaries and across the capillary, cellular and mitochondrial membranes. The PO2 of venous blood (PvO2) depends on the metabolic requirements of the tissues, the blood flow and the shape of the oxygen dissociation curve. It is the lowest blood value in this cascade. This is illustrated schematically in Figure 1.3 (142). PO2 drops slightly in the airways as the ambient air is humidified. A larger drop occurs in the alveoli which is dependent on the balance between oxygen removal by the pulmonary capillary blood and oxygen supply by the alveolar ventilation. Due to ventilation/perfusion inequalities among alveoli and some shunting of blood from the bronchial and coronary venous circulations, arterial PO2 (PaO2) is less than alveolar PO2

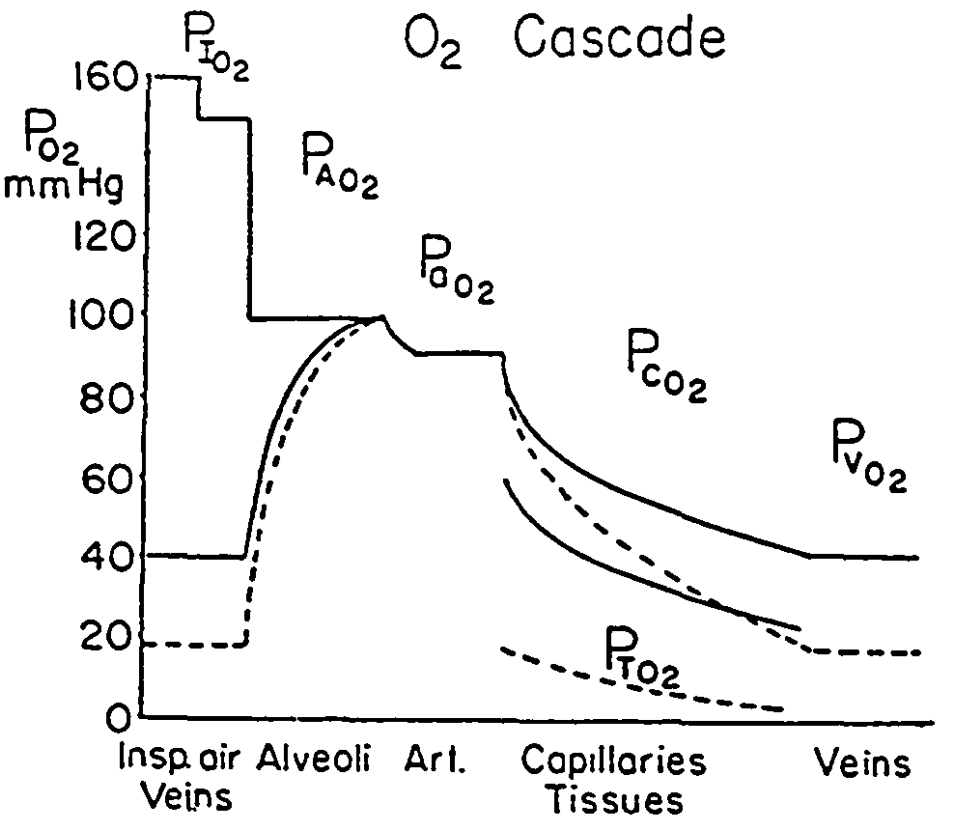

Figure 1.3. Diagram of P_{O_2} from ambient air (PI_{O_2}) to tissue (PT_{O_2}) at rest (*solid line*) and during intense exercise (*dashed line*). Insp. air, inspired air; Art., arterial. See text for details. Reprinted from Stainsby et.al.Med. Sci. Sport Exerc. 20(3):213-221, 1988 with permission.

(PAO2). At the tissue level, oxygen diffuses into the tissue from the capillaries and the tissue PO2 (PTO2) is lower than the capillary PO2 (PcO2) due to uptake of oxygen by the mitochondria. This difference also depends on the diffusion distances and mean transit time.

During severe exercise in normal individuals (dotted lines in Figure 1.3), there is an increase in PAO2, due to a relative increase in alveolar ventilation. This widens the PAO2-PaO2 gradient. The venous oxygen concentration is much lower due to the higher oxygen consumption. The redistribution of blood flow to the working muscles further widens the PaO2-PvO2 gradient because a greater fraction of blood returns to the heart from areas with a low PvO2. The PcO2 to PTO2 difference is similar in spite of the higher oxygen consumption because of reduced diffusion distances due to capillary recruitment (176).

Therefore, possible limiting steps during maximal exercise are: 1) the convective transfer of oxygen from the atmosphere to the alveoli; 2) the diffusive transfer of oxygen from the alveoli to the red blood cells in the pulmonary capillaries; 3) the convective transfer of oxygen via the blood circulation from the pulmonary capillaries to the peripheral tissue capillaries; 3) the diffusive transfer of oxygen from the tissue capillaries to the tissue cell to the mitochondria (120).

Body Size

One approach toward identifying the limiting step in the oxygen transfer cascade, particularly during maximal exercise, has been to study structure as it relates to function in the respiratory and cardiovascular systems of mammals both within and among the huge spectrum of body sizes. Taylor and Weibel (159) proposed the hypothesis of symmorphosis which states that structures supporting a function should be no larger than required by the demand of the limit of performance. This assumes that structures involved in oxygen transport are economically designed, as a result of regulated morphogenesis, to meet but not exceed their maximal functional needs.

In their first series of studies, Weibel, Taylor and colleagues made use of large differences in oxidative capacity that exist between mammals of different body size. They measured maximal oxygen consumption ($\dot{V}O_2max$) in a large number of mammals with body mass varying from 72 grams to 263 kilograms and found that $\dot{V}O_2max$ varied with the 0.8 power of body mass. They then used these body size dependent variations in $\dot{V}O_2max$, in a variety of species, to test for simple correlations between maximal rates of oxygen consumption and single structural parameters determining flow at each step in the oxygen transport cascade. They found a high correlation between the amount of structure present and $\dot{V}O_2max$ with respect to both the mitochondria (85) and the capillaries (59) in skeletal muscle. Maximal cardiac output was found to have

the same scaling factor as $\dot{V}O_2max$ relative to body mass and the arterio-venous oxygen difference was found to be invariant with body size, reflecting a similar oxygen carrying capacity of the blood across body size. However, pulmonary diffusing capacity scaled approximately linearly with body mass rather than $\dot{V}O_2max$. This capacity represents the conductance of the lung for oxygen transfer from air to blood and is related to the alveolar and capillary surface area, capillary blood volume and harmonic mean thickness of the tissue and plasma barriers (174). It appears then that the symmorphosis hypothesis was not supported at the level of the lung. The excess diffusing capacity could be a safety factor to allow for changes in the external environment.

The same authors then examined the adaptive variations in the oxygen transport system of species that are the same body size but have $\dot{V}O_2max$ that differ by 2-3 fold (175). They compared the relatively "athletic" dogs and ponies to "normal" goats and calves of the same respective body size. The volume of mitochondria was still proportional to $\dot{V}O_2max$ (58) and so was the capacity of the muscle microvasculature for oxygen delivery i.e. the product of capillary volume and red cell concentration (20). Two structural parameters, heart size and hemoglobin concentration, contribute almost equally to the 2.5-fold higher convective transport of oxygen in the athletic species (65). The finding that the heart rate of the athletic and normal species pairs are the same at $\dot{V}O_2max$, and that

these are maximal rates predicted by body size, suggested that only an increased stroke volume would increase maximal cardiac output. The authors suggested that all four species operate close to their structural limits for convective oxygen transport in the circulation at $\dot{V}O_2max$ since they fully exploit the possible mechanisms for increasing both cardiac output and the arterial-mixed venous oxygen content difference for the size of their hearts and the concentration of hemoglobin in their blood (65).

The athletic species were found to have a 1.5 times greater pulmonary diffusing capacity than the non-athletic species, which explains only half of their 2.5 times greater $\dot{V}O_2max$. Again the test of symmorphosis failed at the lung. This may be because an increase in pulmonary diffusing capacity due to a higher oxygen uptake can then occur without the need to build more lung structure. As well, symmorphosis depends on regulated morphogenesis which may not be possible in the lung (160).

These studies make two major points: 1) Body size influences both resting and maximal oxygen consumption. This has been examined by others in obese and lean humans. Similar absolute peak oxygen consumption ($\dot{V}O_2$) has been reported in moderately obese and lean controls during both weight supported and weight bearing exercise (23,131,132). Absolute peak oxygen consumption would not be expected to vary even following a large change in body mass if lean body mass is

maintained (93). However, obese individuals have a decreased exercise capacity when expressed as peak oxygen consumption per kilogram of body weight and this would be expected to improve with weight loss. Therefore, even within a species i.e. humans, a substantial increase in body size negatively affects mass specific $\dot{V}O_2max$.

2) Weibel, Taylor and associates conclude that $\dot{V}O_2max$ is ultimately limited by the ability to increase the mitochondrial density in skeletal muscle. However, they also point to the delivery of oxygen via the circulation as a potential limiting step to increasing maximal oxygen consumption. Both these views will be examined further in the next sections.

Cardiovascular Limitations

The Fick equation cited previously states that steady state whole body oxygen consumption is equal to the product of cardiac output and arterial-mixed venous oxygen content difference. The experimental approach to examining which of these variables may pose a limit to maximal oxygen consumption has focused on the change in $\dot{V}O_2max$ induced by physical training and detraining or acute alteration of the blood oxygen capacity.

Attempts to explain the adaptive increase in $\dot{V}O_2max$ due to dynamic exercise training led to controversy regarding whether it was primarily due to central cardiac adaptations, which result in increased oxygen delivery, or to the increased

ability of peripheral working muscle to utilize oxygen. Early training studies showed that a substantial reduction in submaximal heart rate could be achieved without a change in cardiac output (15). Since cardiac output is the product of heart rate and stroke volume, the latter must be higher, for a given absolute $\dot{V}O2$, in the trained individual. Maximal heart rate was similar in trained and untrained subjects, thus the conclusion was drawn that the ability of the heart to increase stroke volume was the limiting factor to $\dot{V}O2max$ (130). The idea fit with observations that heart size in athletes were positively correlated with $\dot{V}O2max$ (117).

Clausen and associates (18) compared exercise responses with trained versus untrained muscle groups after training of either arms or legs in two groups of subjects to distinguish between cardiac and peripheral muscle adaptations to training. Only a small decrease in submaximal heart rate during leg exercise after arm training was observed whereas a larger decrease was seen during arm exercise following leg training. The decrease in heart rate during exercise with untrained muscles was similar at both moderate and severe submaximal workloads and reflective of that seen at rest. The authors did not speculate on the mechanism for the bradycardia but suggested it was different from bradycardia during exercise with trained muscles, which exceeded resting heart rate changes and was greater at higher work rates. They proposed that alterations in the trained muscles and central

circulatory changes both contribute to the effects of physical training on the circulation. $\dot{V}O_2max$ was only measured in three subjects from the leg training group and the authors admit that one of these subjects employed additional muscle groups during the post-training arm exercise test. This surely inflates the reported average increase in $\dot{V}O_2max$ of 9.9% in the untrained arms, which is most likely not significant. However, it has been quoted by Saltin to support the contention that oxygen extraction and utilization could increase without any adaptation of the skeletal muscles (130). Interestingly, this contradicts his own earlier finding of no significant increase in $\dot{V}O_2max$ in the untrained leg in a one-legged training study (129).

Saltin's major contribution to this literature was the well known bed rest detraining and subsequent training study (127). Oxygen transport and body composition were studied in five subjects before and after 20 days of bed rest followed by a 55 day period of intense physical training. They used extensive invasive procedures which involved placement of arterial and venous catheters, muscle biopsies, determination of plasma volume, measurements of diffusion capacity of the lung, blood gases, lactate and electrolytes and dye-dilution cardiac output. Two of the subjects had been previously physically trained and three were sedentary. After bed rest a decrease in $\dot{V}O_2max$ by 28% was accounted for by a 26% decrease in maximal cardiac output and no change in arterial-venous oxygen

difference. Maximal heart rate was unchanged and maximal stroke volume decreased by 30%. The authors attributed the decreased cardiac output to the reduced stroke volume due to either changes in myocardial function or impaired control of capacitance vessels causing a decrease in venous return. They argued that since maximal stroke volume was still decreased by 24% when the effect of "venous pooling" was eliminated by exercise in the supine position, a myocardial impairment was more likely. However, they also reported an average decrease in blood volume from 5.065 to 4.700 litres and a decrease in lean body mass of 1 kilogram but did not consider the effect of these changes on the determinants of venous return. The decrease in maximal cardiac output must mean that venous return was decreased. If the heart was involved in the decrease in venous return then right atrial pressure must have increased, however this was not measured. The principal circuit factors include a decrease in mean systemic pressure, due to decreased stressed vascular volume or possible increase in venous compliance, and a smaller fraction of blood flow being redistributed to the reduced amount of skeletal muscle which is the fast time constant bed. All changes were reversed with training, although, interestingly, the increase in VO2max of 20-30% was complete after 30 days of training, and did not increase further in either sedentary or previously active subjects. This may suggest a physiological limit to training.

The idea that the ability of the heart to increase stroke

volume limits cardiac output during maximal exercise suggests that stroke volume is a controlled variable during exercise. This is unlikely since both heart rate and cardiac output are tightly controlled in proportion to $\dot{V}O_2$ by neural and metabolic activity, which will be discussed later in section III. Therefore, the increase in stroke volume during exercise following physical training is secondary to the fact that cardiac output is greater at any heart rate after training (17). This agrees with the idea of the heart as a force-fed pump which pumps out only what it receives. This output can be accomplished over a wide range of heart rates due to the adaptability of the heart for adjusting stroke volume. However, heart size may constitute a structural limit. This was demonstrated in two species of animals where the heart was "enlarged" by pericardectomy and an increase in maximal stroke volume, cardiac output and $\dot{V}O_{2max}$ resulted (55,156). Heart size is normally a constant fraction of body size across mammalian species (113), although a larger heart size is present in the athletic vs non-athletic species of the same body size in the Weibel and Taylor studies of adaptive variation in $\dot{V}O_{2max}$, discussed previously (65).

Oxygen delivery to the tissues is the product of cardiac output and the arterial oxygen content. The latter has been manipulated experimentally by altering the amount of hemoglobin through transfusion of red blood cells, or the arterial oxygen saturation by breathing hypoxic gas mixtures.

VO2max was decreased after blood loss (35), acute anemia (177), acute and chronic hypoxia (126,151), and increased following the transfusion of red blood cells (9) and with increased PO2 in inspired air (38). Therefore, oxygen delivery and maximal oxygen consumption are closely matched (130). The convective transport of oxygen cannot be considered the only possible limit to oxygen transport, since each link in the cascade may be shown to limit $\dot{V}O_2max$ under certain specific conditions. For example, although the healthy pulmonary system is thought to have large reserves for gas exchange even during maximal exercise, this has been shown to be exceeded in highly trained athletes who exhibit arterial hypoxemia at extremely high work rates (24). Arguments for and against a limitation to $\dot{V}O_2max$ by factors related to oxygen transfer and utilization within skeletal muscle are presented in the next section.

Skeletal Muscle Limitations

Factors which determine how low the mixed venous oxygen content can be at $\dot{V}O_2max$ include muscle blood flow, diffusion of oxygen from muscle capillary to mitochondria and mitochondrial capacity for oxidative phosphorylation, which includes density of mitochondria as well as enzymatic activity. Each of these has been implicated as a limitation to $\dot{V}O_2max$.

Part of the decrease in mixed venous oxygen content during exercise is simply due to the redistribution of blood flow to

the active muscle bed. This results in a lower oxygen content in a greater fraction of the total venous return, which by itself will lower the mixed venous content. The ability of skeletal muscle to accommodate an increased blood flow at VO2max was suggested by Mellander and Johansson (92) to be limiting, based on known perfusion rates of various organs and tissues during exercise, measured by the ^{133}Xe clearance method. They suggested that cardiac output and peripheral conductance (i.e. the reciprocal of resistance) were well matched at $\dot{V}O2max$. A more recent study has concluded that peak perfusion of the knee-extensor muscle in humans during dynamic one-legged exhaustive exercise can be as high as 200 $ml \cdot min^{-1} \cdot 100g^{-1}$ (3), four times higher than previously thought (47). Blood flow was measured in the femoral vein using a constant infusion thermodilution method. The authors argue that these results indicate that the capacity of skeletal muscles to accommodate increased blood flow and consume oxygen during exercise when a large fraction of muscle mass is active, far exceeds the ability of the central circulation to increase oxygen delivery (3). This conclusion is not supported in other animal species such as the horse (4) or in the dog, where the increase in mean limb muscle blood flow, measured by the microsphere technique, showed a clear tendency to level off at works loads corresponding to 70% of $\dot{V}O2max$ (107). In the human study, the authors assumed that the small muscle mass studied was representative of all muscles engaged in dynamic exercise

in terms of metabolic capacity and recruitment patterns. They also reported a high femoral venous oxygen content at the high blood flows. This may indicate that the flows measured in the femoral vein included flow from other regions than the active muscle but this would not account for the unusually high flows measured. The authors speculated that the transit time necessary for peripheral diffusion may have been exceeded at high flows which resulted in a higher venous oxygen content. In spite of this, the study demonstrates that muscle blood flow and metabolic activity can reach higher levels than originally thought in humans and is unlikely to limit VO2max under normal conditions. That this automatically indicates that the pump capacity of the heart is the major limiting factor in maximal exercise employing large muscle groups, as advocated by Saltin (130) and Rowell (119,120,124) is arguable and leads to a circular reasoning. As discussed in section I, cardiac output is determined largely by peripheral vascular factors affecting venous return. Redistribution of blood flow to the muscle bed during exercise is an important factor and itself reflects metabolic vasodilation of the muscle bed as well as active vasoconstriction in inactive beds. Therefore, does cardiac output limit muscle blood flow during maximal exercise or the reverse? This will be discussed further with control mechanisms in section III. First, other potential limiting factors at the muscle level will be considered.

Evidence for a diffusion limitation of oxygen between the

hemoglobin molecule in the red blood cells of the muscle capillary and the muscle mitochondria during maximal exercise was put forward by Wagner and colleagues who suggested that for a given level of convective oxygen delivery, muscle oxygen diffusing capacity limits $\dot{V}O_2max$ (167). This is based on a study which examined the relationship between femoral venous PO_2 and VO_2max in healthy subjects as inspiratory PO_2 was altered during cycling exercise. Average muscle capillary PO_2 was calculated by Bohr integration. The authors found that both femoral venous and muscle capillary PO_2 were linearly related to $\dot{V}O_2max$ with an intercept not different from the origin. The slope of this relationship illustrated Fick's first law of diffusion which is $\dot{V}O_2 = DO_2 (PcO_2 - PmitoO_2)$; where DO_2 is a lumped sum diffusing capacity, PcO_2 is mean capillary PO_2 and $PmitoO_2$ is mitochondrial PO_2 which is assumed to be 0 at maximal work. Therefore, $\dot{V}O_2max$ must decline when venous PO_2 is lowered because the pressure gradient from the capillary to the mitochondria is decreased and less oxygen is able to diffuse per unit time from the red blood cell to the mitochondria in muscle (116). This group were also able to separate the relative roles of oxygen delivery and oxygen diffusion as determinants of $\dot{V}O_2max$ in an animal study where they kept oxygen delivery constant to maximally contracting isolated canine gastrocnemius muscle and compared low blood flow/high CaO_2 and high blood flow/low CaO_2 conditions. In support of the hypothesis of diffusion

limitation, VO2max of this muscle model and venous PO2 were both significantly higher in the low flow/high CaO2 condition. Therefore, $\dot{V}O_2max$ is not uniquely dependent on oxygen delivery (56). In these studies, the authors have assumed a simple capillary geometry and a homogeneous blood flow without shunts in maximally working muscle (167). They also assume proportionality between whole body $\dot{V}O_2max$ and leg $\dot{V}O_2max$ which was supported by a subsequent study in humans during cycling exercise (69). These studies highlight the critical importance of diffusive oxygen transport from muscle capillary to mitochondria in determining $\dot{V}O_2max$, while at the same time recognizing the potential limits of other steps in the oxygen transport system.

Weibel and Taylor, whose work was discussed earlier, raised the question of whether the volume of skeletal muscle mitochondria represented a structural limit to $\dot{V}O_2max$ (160). This was suggested by their studies which showed that this variable increased in direct proportion to $\dot{V}O_2max$ both among mammals of different body size (85) as well as between athletic and non-athletic species of similar body size (58). Taylor concluded that the mitochondria are an invariant building block in that more are added to achieve a higher $\dot{V}O_2max$ (160). This evolutionary approach is difficult to compare with training or cross-sectional studies within a species which also examine adaptive variation in $\dot{V}O_2max$, since the changes in VO2max differ by many orders of magnitude.

However, increased mitochondrial volume and increased activity of various oxidative enzymes have been reported in humans and animals as a result of dynamic exercise training (44,57,100,128) which supported the metabolic capacity of skeletal muscle as the major limit to $\dot{V}O_2max$. This fit with the finding of no increase in $\dot{V}O_2max$ in the untrained leg in one-legged training studies (129). However, when arm exercise is added during leg exercise at $\dot{V}O_2max$, effectively recruiting more muscle mass (and mitochondria), an increase in $\dot{V}O_2max$ was not found (134). This, along with the studies cited earlier which noted an increased $\dot{V}O_2max$ with increased oxygen delivery, are taken as evidence against a metabolic limit to VO_2max imposed by restricted oxidative capacity of skeletal muscle mitochondria (120). In addition, metabolic improvements in skeletal muscle in humans, a result of physical training, appeared to be a more important factor in the improvement in endurance capacity, through alterations in substrate utilization, rather than $\dot{V}O_2max$ (128).

Summary

The answer to what limits $\dot{V}O_2max$ in healthy subjects is still controversial and has served to stimulate research efforts over the past century. Much of this has been applied in a clinical setting to improve the functional capacity of patients who have pathological abnormalities in one or more steps of the oxygen transport cascade. In some cases, these patients can serve as models for examination of the relative

importance of these steps during peak exercise.

It is evident from this brief review that there is data to demonstrate that each step in the oxygen transport path may pose a limit to $\dot{V}O_2max$ under certain conditions. Therefore an integrated approach to the issue has been taken by some authors. Wagner suggested that the sensitivity of $\dot{V}O_2max$ to changes in the transport capacity of any one step may vary such that correlations of different degrees may be observed as different components are altered (167). di Prampero presented a multifactorial mathematical model based on the conductance of oxygen at the various steps in the transfer pathway and the relative change in $\dot{V}O_2max$ seen in previous studies compared to the change in various functional parameters which he equated with the oxygen conductance at each step. While he discounts the potential limitation at the level of the lung, this analysis highlights the interdependence of all steps and concludes that during two-legged exercise about 75% of $\dot{V}O_2max$ is set by convective oxygen delivery and the remaining fraction is equally partitioned between peripheral perfusion and diffusion and mitochondrial utilization capacity. During one-legged exercise, the limits are equally set by oxygen flow to the muscle and transport and diffusion within the muscle (27,28).

Thus, maximal dynamic exercise involves a integrated response of all systems involved in oxygen transport. Changes in $\dot{V}O_2max$ may be most sensitive to alterations in oxygen

delivery. The increase in cardiac output during exercise, which must occur as a result of peripheral vascular and to a lesser extent cardiac changes, is a major factor in oxygen delivery and is somehow precisely matched to the level of oxygen consumption. The possible regulatory mechanisms involved will be discussed in the next section.

C. SIGNALS CONTROLLING CARDIOVASCULAR RESPONSE TO EXERCISE

There has been extensive work on the signals that control the cardiovascular response to dynamic exercise. A combination of neural, humoral and mechanical influences alter heart rate, blood pressure, cardiac output and blood flow distribution during exercise to match the increase in metabolic needs. The specific work factor that stimulates the coordinated response of the heart and resistance and capacitance vessels is still a matter of debate. In this section, I will review the central, local muscle afferent, baroreceptor and humoral factors and the role of the muscle pump in the control of this response.

The efferent pathway for the hemodynamic changes resulting from these signal inputs during dynamic exercise occur through the autonomic nervous system, whereby there is a general decrease in parasympathetic outflow to the heart and an increase in sympathetic activity to the heart and peripheral vasculature. However, the well known precise matching of

cardiac output to whole body oxygen consumption during progressive dynamic exercise is maintained despite cardiac denervation in animals and humans (12,16,32,61). This suggests that autonomic neural efferent outflow to the heart is not required for the increase in cardiac output during exercise and emphasizes the importance of peripheral vascular factors.

Neural Control during Dynamic Exercise

Since the nineteenth century it has been recognized that two mechanisms of neural control play an important role in the precise matching of the cardiovascular response to the intensity of activity in dynamic exercise. They are a feedforward system termed "central command", whereby neural signals descending from the motor cortex "irradiate" to and stimulate the cardiovascular centres in the brain stem, (37,73) and a feedback system whereby mechanical and metabolic signals from the contracting muscle stimulate muscle afferents which relay to the same cardiovascular centres in the medulla, thereby altering autonomic nervous outflow (1,21,97). The relative contribution and interaction of these with other reflex mechanisms, such as the arterial baroreceptor, and the response of heart rate, blood pressure and cardiac output varies according to whether the exercise is static or dynamic, and is still somewhat controversial (98,120). Studies of dynamic exercise are few but are the focus of this review since this type of exercise employs a large fraction of muscle mass and demonstrates a close matching of cardiac output and

oxygen consumption. Studies which use static or isometric exercise will be referred to where relevant.

The concept of centrally generated motor command signals irradiating to medullary cardiovascular control centres appeared in early studies to explain the immediate increase in heart rate and ventilation at exercise onset (73). The term "cortical irradiation" was used by Krogh to describe a diffuse outflow of motor impulses from the cerebral cortex to the cardiovascular centres in the medulla, rather than discrete neural pathways. Later studies in animals confirmed that electrical or chemical stimulation of the subthalamic locomotor region in the rostral brain, which results in locomotion, also causes a cardiovascular response, even in a paralyzed animal (37,168). There is a differential effect on sympathetic outflow to various vascular beds such that blood flow to heart, diaphragm and limb muscles increases and flow to the kidneys decreases during stimulation. Heart rate, arterial pressure and left ventricular systolic pressure and rate of pressure development all increase (168). In human studies, central command can be enhanced during dynamic exercise by applying neuromuscular blockade to induce muscle weakness. The subject then requires more effort at a given power output. This type of study has shown little evidence of increased effort on heart rate and blood pressure responses during moderate exercise (43), although responses are increased with increased central command during isometric

contractions (75). Victor and associates (165) found that heart rate and muscle sympathetic nerve activity (MSNA) were dissociated during both contralateral graded dynamic handgrip exercise and arm cycling, consistent with the view that central command appears to initiate parasympathetic withdrawal and tachycardia at the onset of exercise whereas stimulation of chemically sensitive muscle afferents activates sympathetic outflow to non-exercising muscles as well as the heart at moderate exercise levels (164,165).

The suggestion was made in the late nineteenth century that cardiovascular responses during exercise are due to a reflex originating in skeletal muscle (62,180). The first major experimental evidence for this was the work of Alam and Smirk (1,2) in the 1930's. They found that heart rate and arterial pressure increase in human subjects during intermittent muscle contractions with local circulatory occlusion and this persisted after exercise as long as the occlusion was maintained. They concluded that receptors in working skeletal muscle were activated by metabolites produced by muscle contraction and caused a reflex increase in heart rate and blood pressure. Vascular occlusion prevented the clearance of these metabolites and maintained the stimulus to muscle receptors. Asmussen and Nielsen (5) found similar exaggerated heart rate and blood pressure responses during submaximal cycling exercise when blood flow to the legs was occluded with cuffs. However, oxygen consumption was reduced by 50% during

exercise with occlusion compared with normal circulation. Interestingly, cardiac output was similar in both groups indicating that it was related to work intensity rather than $\dot{V}O_2$. It is possible that an increase in cardiac output during occlusion, due to stimulation of muscle afferents, may have been counteracted by the alteration in blood flow redistribution to active muscle which is an important determinant of venous return. This was not recognized by the authors but is a possible explanation for the lack of change in cardiac output and does not rule out control by muscle afferents.

The reflex nature of the exercise pressor response was established by Coote and colleagues who abolished the increase in arterial pressure during electrical stimulation of the distal end of cut ventral roots in cats by cutting the dorsal roots (21). Finely myelinated type III and unmyelinated type IV afferent fibres in skeletal muscle were shown by differential blockade studies in animals to be the afferent limb of the muscle reflex during muscle contraction (88). Using direct nerve recordings, Kaufman demonstrated that type III fibres respond predominately to mechanical stimulation whereas type IV fibres are activated mainly by chemical stimuli, although there is considerable overlap (66,67). This resulted in the use of the terms muscle mechanoreceptors and chemoreceptors. A role for large myelinated type I and II afferent fibres was ruled out by differential blockade studies

(88) and the lack of reflex changes in cardiovascular variables in response to muscle vibration (87).

The muscle reflex was studied during dynamic exercise mainly by employing ischemia to exaggerate the cardiovascular response or by blocking type III and IV muscle afferents to attempt to reverse it. Rowell and colleagues studied the effect of brief periods of graded ischemia on cardiovascular variables and ventilation during dynamic exercise as well as recovery. They showed that the blood pressure that is maintained after exercise is directly related to the degree of ischemia. Heart rate increased only slightly during occlusion during exercise, possibly due to inhibitory effects of the arterial baroreceptor, and, in contrast to blood pressure, decreased normally during recovery (122). The authors speculated on a threshold of activation of muscle afferents during dynamic exercise related to a decrease in muscle perfusion below some critical level. Muscle perfusion decreases during static exercise since strong isometric contractions effectively block blood flow to the exercising muscle and elicit a large increase in blood pressure (88). This exercise pressor response was enhanced by ischemia which augmented increases in muscle PCO2, lactate and decreases in pH (148). These are among some of the metabolites proposed to signal the metabolic error and stimulate chemically sensitive muscle afferents.

Wyss and colleagues investigated whether the muscle

"chemoreflex" was tonically active or dependent on reductions in muscle perfusion to some critical level during dynamic exercise. They induced stepwise partial occlusions of the terminal aorta in dogs which exercised at various intensities on a treadmill. During mild exercise, there was no change or a slight decrease in heart rate and cardiac output and no change in arterial pressure during progressive occlusion until terminal aortic flow was decreased by 2/3, whereupon all variables increased sharply. The reduction in hindlimb perfusion necessary to invoke a powerful pressor response was much smaller with increasing work loads so that at the highest load examined, which was still a relatively moderate intensity for dogs, no threshold was observed and all variables increased linearly in response to graded occlusions. The authors concluded that at low levels of dynamic exercise, flow-dependent metabolic signals from working muscle are not likely to be the primary drive for increases in heart rate and blood pressure but at higher exercise levels, this reflex may play an important role in matching cardiovascular responses to the metabolic activity of skeletal muscle. The pressor reflex had a gain of 1 after correction was made for increases in arterial pressure due to the mechanical effects of occlusion (178). Subsequently, Sheriff et.al. from the same group, showed that the threshold for activation of the muscle chemoreflex during exercise with progressive occlusions in dogs was related to a critical level of oxygen delivery, below

which blood pressure increased. This increase was closely related to increases in femoral venous lactate and decreases in pH (138). In addition, arterial baroreceptor denervation doubled of the gain of the reflex pressor response indicating that the arterial baroreceptor opposed the pressor response to occlusion during exercise (136).

Whereas the latter two studies focused primarily on blood pressure responses, the initial finding of a decreasing threshold of activation of reflex changes in heart rate and cardiac output with increasing work rates during dynamic exercise suggests that feedback from muscle afferents may play an important role in matching cardiac output to exercise intensity. A proximal cold nerve block was used by Tibes (161) to interrupt feedback from type III and IV muscle afferents during 5 minutes of dynamic pattern sciatic nerve stimulation in anesthetized dogs. Cooling the nerve to 1 degree C effectively blocks afferent transmission from type III and IV muscle afferents (42). The increases in heart rate and ventilation seen during contractions without blockade were abolished by the cold block. As well, mean arterial pressure was decreased and contraction-induced increases in oxygen consumption and femoral flow were blunted. Comparison of work with one leg and two legs revealed that increases in these variables during dynamic work without blockade were related to the amount of active muscle mass. After prolonged stimulation of 14 minutes with cold block, heart rate became slightly

elevated and arterial pressure returned to control values. The authors speculated that this was due to bloodborne catecholamines. The time course of change in heart rate and femoral blood flow was similar at exercise onset, indicating that the factors controlling heart rate and blood flow are related to similar metabolic events. Although cardiac output was not measured, this study suggests that, in the absence of central command, stimulation of type III and IV muscle afferents can increase the cardiovascular responses observed during dynamic exercise.

Afferent feedback from the exercising muscle has been blocked in humans using epidural anesthesia in order to eliminate feedback from type III and IV muscle afferents. During graded dynamic exercise to exhaustion with epidural anesthesia, Fernandes et.al.(40) showed that the heart rate response was the same as exercise without block whereas blood pressure was markedly attenuated. They concluded that the muscle chemoreflex is necessary for blood pressure but not heart rate control during dynamic exercise. Strange et.al.(155) recently observed that the blood pressure response to dynamic voluntary and electrically stimulated (no central command) leg extension exercise was abolished during electrically stimulated exercise with epidural anesthesia (both central command and muscle reflex inoperative). Therefore input from muscle afferents is essential for a normal blood pressure response to dynamic exercise. However,

heart rate and cardiac output responses were only slightly decreased by the epidural anesthesia condition, although this could be due to the influence of the arterial baroreceptor. Although only low levels of exercise were studied, this work raises the question of how neural mechanisms interact to produce the measured heart rate and cardiac output responses which are so closely matched to dynamic exercise intensity.

On the basis of studies with conscious humans, many authors concluded that the muscle chemoreflex controls blood pressure responses during dynamic exercise but is not essential for heart rate or cardiac output control (2,40). However, this conclusion has been challenged by others who propose that the influence of the arterial baroreceptor may mask the effect of the muscle reflex on these variables (122,123,155), much as it was found to oppose the pressor response to occlusion during exercise by Sheriff and others (136). This interpretation assumes that the arterial baroreceptors are reset during dynamic exercise. Indirect evidence for a centrally-mediated resetting of the arterial baroreceptor at the onset of exercise is evident in animal studies using either reversible isolation of the carotid sinuses to maintain a constant carotid sinus pressure or vasodilators to keep arterial pressure constant during dynamic exercise (29,169,170). Melcher and Donald (91) reported that the arterial pressure at which the carotid baroreflex operates is progressively elevated as the work load is increased. However, the shape of

the stimulus-response curve; maximal gain, threshold and saturation pressures are unchanged during exercise (91). Stimulation of muscle afferents caused a redistribution of cardiac output to active muscle in anesthetized vagotomized dogs, when carotid sinus pressure was maintained constant (19). Heart rate and cardiac output increased normally during exercise with carotid sinus pressure maintained at normal levels in conscious dogs with aortic baroreceptor denervation, although a severe hypertension was observed (169). This result may be interpreted in two opposing ways: 1) heart rate and cardiac output increases during moderate levels of dynamic exercise are stimulated by an exercising muscle reflex with no influence from the arterial baroreceptor; 2) as a result of a reset of the arterial baroreceptors at exercise onset, the carotid sinus stimulus drives the heart rate response since their constant pressure would be perceived centrally as a progressively increasing hypotensive stimulus. A recent study in humans exercising up to 75% of VO2max demonstrated an upward shift in the carotid sinus stimulus-response curve as work rate increased (105). These authors manipulated carotid sinus pressure non-invasively by a neck suction device and assumed a constant interaction between carotid sinus and intact aortic baroreceptors. There was no effect of exercise on the slope of the heart rate/carotid sinus pressure or mean arterial pressure/carotid sinus pressure relationships, in agreement with the previously cited animal work. Therefore,

there is strong evidence in favour of a resetting of the arterial baroreceptor during dynamic exercise.

Rowell has formulated a hypothesis of cardiovascular control which integrates the influence of this baroreceptor resetting during exercise with the central and peripheral neural signals (120,123). He postulates that central command is operative at the onset of dynamic exercise and results in an increase in blood pressure, heart rate and cardiac output through withdrawal of parasympathetic tone (163) and immediate resetting of the arterial baroreceptor (60). A time lag occurs until arterial pressure is increased to the new operating point by increases in cardiac output and provides a pressure error signal. This error signal is sensed by the arterial baroreceptor which results in activation of sympathetic outflow and results in vasoconstriction in non-active regions. This further increases heart rate and cardiac output. The range of parasympathetic control of the heart may vary in different species, and could determine the level of exercise at which activation of the sympathetic nervous system first occurs. The muscle chemoreflex becomes tonically active during moderate to severe exercise as a result of a mismatch between muscle blood flow and metabolism which stimulates muscle afferents. When vascular conductance exceeds cardiac pumping capacity in the most severe exercise, both chemoreflex and baroreflex must maintain blood pressure by vasoconstricting active muscle. The chemoreflex is thought to become more

dominant as exercise intensity increases since its strength is a function of muscle mass (121). However, it is opposed by the baroreflex and this opposition could be stronger or weaker with increasing work rate, depending on how much the baroreflex function curve has shifted and whether the operating point is on a more or less sensitive region of the curve (123). Therefore, this argument favours a blood pressure error, or a mismatch between cardiac output and vascular conductance, as the primary feedback stimulus. A flow error, or mismatch between muscle blood flow and metabolism, is considered a secondary signal which occurs at higher levels of exercise. This view assumes that neural control of the heart is the main influence on cardiac output responses and that cardiac pumping capacity is exceeded by vascular conductance at maximal exercise. It also does not explain the linear relationship of cardiac output to oxygen consumption during dynamic exercise. In particular, the peripheral vascular factors affecting venous return and their role in increasing cardiac output in proportion to metabolic demand during dynamic exercise have not been considered in this scheme.

Deschamps et.al.(26) outlined the peripheral mechanisms by which cardiac output is increased during carotid sinus hypotension. They showed that a decrease in carotid sinus pressure resulted in a decrease in total venous capacitance, primarily due to decreased unstressed volume and venous resistance in the splanchnic region. This would lead to an

increase in vascular stressed volume in the intact circulation and increase venous return and cardiac output.

The muscle afferents involved in inducing autonomic reflex adjustments of both resistance and capacitance vessels were examined by Webb-Peploe et.al. (171). They observed that injection of capsaicin decreased splenic capacitance (assessed by changes in splenic venous pressure) to a similar extent as electrical stimulation of hindlimb muscle afferent nerve fibres. This suggests that capsaicin-sensitive skeletal muscle receptors are normally activated by muscular exercise to cause changes in capacitance which would affect redistribution of blood flow. These studies point out the importance of neurally-mediated changes in the peripheral vasculature on cardiac output as compared to stimulation of the heart itself. This agrees with the results of denervation studies in animals and heart transplant patients where the cardiac output/oxygen consumption relationship is maintained despite cardiac denervation (12,16,32,61). In addition, in dogs where the functional nervous system was destroyed by decapitation, there was little difference in control of cardiac output in response to stimulated muscle contraction compared with intact dogs. This contrasts with the dependence of arterial pressure control on an intact nervous system (30). Therefore, although the literature points to an integrated neural control of heart rate and arterial pressure employing both feedforward and feedback signals during dynamic exercise, it is not clear to

what extent cardiac output is controlled by these neural influences.

Humoral Factors

Various humoral agents may contribute to or modify the extent of the cardiovascular response to dynamic exercise. Vasodilator substances include those released as by-products of the metabolic activity involved in muscle contraction and those released from the endothelium. Vasoconstrictor peptides are released from nerve endings, the endothelium or in response to changes in osmolality and plasma volume which occur during dynamic exercise. These agents act locally on vascular smooth muscle and may interact with other humoral and neural factors. The resulting changes in local blood flow influence the overall redistribution of blood flow during exercise.

Substances such as hydrogen and potassium ions, inorganic phosphate, carbon dioxide, adenosine, adenosine nucleotides, lactate, intermediates of the tricarboxylic acid cycle, prostaglandins and the state of hyperosmolarity or decreased PO2 appear in the interstitium during muscle contractions and have direct and indirect vasodilator actions. Each of these, at one time, was thought to be the primary stimulus for exercise-induced vasodilation but a multifactorial metabolic control system is more likely since each factor lacks any sustained influence as contraction continues (135). Recently this field has expanded to include endothelium-derived

relaxing factor (EDRF) or nitric oxide (86). In addition, an endothelium-dependent flow-mediated vasodilation has been demonstrated in isolated canine femoral artery in vivo (111) and is thought to be mediated by nitric oxide and/or prostacyclin in response to changes in shear stress at the arterial wall (141). This potent local regulatory mechanism provides a means by which blood flow effectively influences vascular tone over a wide range of flows and could potentially play a role in maintaining muscle blood flow during exercise following initial metabolic vasodilation.

Plasma concentrations of potent vasoconstrictor peptides such as arginine vasopressin, neuropeptide Y and endothelin-1, among others, are increased during conditions associated with sympathetic activation (11,79,90,101,162,166). This includes dynamic exercise, except for plasma endothelin-1 levels which have not been reported (90,179). However, endothelin-1 release from endothelial cells occurs in response to various vasoactive substances and stimuli such as hypoxia (115), increased shear stress and chronic increases in blood flow (94,96), as occurs with EDRF and prostaglandins. The balance between endothelial release of relaxing and contracting factors determines the effect on local vascular tone.

Vasopressin is released in response to increased plasma osmolality and decreased plasma volume, both of which occur during dynamic exercise (166). Blockade of vasopressin 1 receptors in exercising miniswine resulted in a decrease in

mean arterial pressure and an attenuated vascular resistance in splanchnic area vessels, which demonstrates an effect of circulating vasopressin on distribution of cardiac output during exercise (149).

Neuropeptide Y is co-released with norepinephrine from sympathetic nerve endings during moderate to severe exercise but follows a slower time course (110). In pithed rats, exogenous neuropeptide Y produced an increase in cardiac output with no change in heart rate and increased total peripheral resistance mainly by increasing renal and mesenteric vascular resistance (81). The same authors showed a similar effect with exogenous endothelin-1 (82). Deschamps et.al. demonstrated, in dogs, that the mechanism for the neuropeptide Y-induced increase in venous return was a decrease in splanchnic vascular capacitance due to decreased unstressed volume in this region (25). This suggests a role for neuropeptide Y in the control of peripheral vascular, particularly venous, tone which could influence cardiac output and its distribution.

Interactions between neural and humoral control of vascular tone likely modulates peripheral vascular changes during exercise. Both arterial baroreceptor hypotension and muscle chemoreflex activation can increase plasma vasopressin levels (63,104,114,173). Factors released from endothelial cells can act in a paracrine manner to modulate arterial baroreceptor sensitivity (13,14). Changes in sympathetic outflow via the

arterial baroreceptor may exert a direct neural effect on peptide release, as seen with vasopressin, or an indirect effect via local changes in perfusion pressure or blood flow in different regional vessels. The effect of alterations in baroreceptor activity on plasma neuropeptide Y and endothelin-1 levels are not known. The sympathetic neurotransmitter norepinephrine can affect responses of the endothelium. As well, both endothelial-derived factors and neuropeptide Y can alter release of norepinephrine (22,80,95). These complex interactions are difficult to study in the intact animal or human but may contribute to changes in regional blood flow during exercise stress which in turn influence venous return.

Mechanical Effect of Muscle Contraction

The role of the muscle pump in circulatory control during exercise has received increasing attention in the last 10 years. Early work demonstrated that blood volume and venous pressure in the lower limbs of humans is decreased by muscle contractions (78,112). It has been suggested that this may serve 3 functions: 1) promote blood volume transfer centrally by "pumping" blood from the venous reservoir back to the heart and prevent venous pooling (74,120); 2) increase the effective perfusion pressure gradient across the muscle vascular bed and thereby increase muscle perfusion (41,74,77,120,150); and finally 3) protect muscle capillaries from increased venous pressure during the contraction phase and reduce fluid filtration (102,153).

The term muscle pump originated with the idea that rhythmic muscle contractions could act to repeatedly compress muscle veins and thereby empty them of blood. The blood is then propelled toward the heart. Competent venous valves prevent any backflow (112,150). The concept that muscle contractions mechanically push venous blood in the dependent limbs back to the heart is a common one and depends on the presence of one-way venous valves (120). Bevegard and Lodin (8) examined the effect of congenital absence of venous valves on stroke volume in 5 patients during supine and upright exercise with right heart catheterization. These patients had higher heart rates and lower stroke volumes than normal subjects, when they moved from supine to the sitting position and during upright exercise, although cardiac output was relatively normal. The decreased stroke volume was attributed to the lack of ability to redistribute blood from the legs to the central circulation due to lack of valves, however it most likely reflects the higher heart rate in patients compared with normals, which results in a normal cardiac output response during exercise. The heart rate differences between groups could have resulted from differences in physical training or sympathetic activation between patients and normals. Interestingly, right ventricular end-diastolic pressure decreased during upright exercise in most patients, compared with no change in normals. If taken as representing right atrial pressure, this indicates a reduction in the volume of blood translocated by muscle

contractions, perhaps due to backflow as a result of the lack of venous valves. The lack of decrease in venous pressure at the ankle would support this interpretation. However, this can not be distinguished from a reduction in right atrial pressure due to the increased heart rate. In any case, venous return was increased during exercise in these patients, resulting in a normal cardiac output response. In view of this, the presence or absence of venous valves seems of questionable significance. However, the mechanical effect of muscle contractions on the determinants of venous return has received little attention.

Changes in mean systemic pressure during muscle stimulation in areflexic anesthetized dogs was measured by Guyton et.al.(52). They found that static contraction of the abdomen and hindlimb muscles resulted in three-fold increases in mean systemic pressure. This was not blocked by sympathetic ganglionic blockade but was blocked by skeletal muscle paralysis. They concluded that mechanical effects of skeletal muscle activity in the abdomen and hindlimb increases mean systemic pressure but they could not distinguish between the recruitment of volume and compliance effects of muscle contractions. This view was supported by Sheriff and colleagues (139) who found that the muscle "pump" was more important than reflex adjustments in capacitance in mobilizing volume in response to an induced fall in cardiac output during mild exercise levels in conscious dogs. This may be due to a

low sympathetic activity at the levels of exercise used in their study. They also found no change in slope in the relationship between central venous pressure and cardiac output (equivalent of a venous return curve). This relationship is affected by the fractional distribution of flow. Thus, the lack of change suggests no increase of fractional flow to the fast time constant muscular bed and no change in resistance to venous return. In fact, the time constant of venous drainage of the isolated gastrocnemius in anesthetized dogs has been shown to decrease during stimulated muscle contractions (103).

In summary, the mechanical effect of muscle contractions promotes rapid increases in venous return, particularly at exercise onset, due to a central shift of blood volume from the capacitance vessels. The effect of this mechanical stimulus on the peripheral vasculature during dynamic exercise has not been completely determined.

The second proposed function of the muscle pump resulted from the work of Folkow (41) who suggested that muscle contractions can mechanically increase the blood flow into muscle. This is based on the theory that a decrease in the pressure in the compliant region of the vasculature at the start of the relaxation phase of repetitive muscle contractions, results from the reduction in volume in this region immediately following the muscle contraction. The decrease in pressure would transiently increase the gradient

for flow into the muscle since arterial pressure is essentially constant and implies a gain in effective perfusion pressure. Folkow's experiments were performed on the hindlimb and isolated gastrocnemius of anesthetized cats. Blood flow was measured with electromagnetic flow probes on femoral artery and vein and indicated an increase in arterial inflow and decrease in venous outflow between contractions and the reverse during contraction, although baseline flows were not shown. Mean flow during exercise was not greater than under "free" flow conditions during the immediate recovery period. As well, since maximal vasodilation of the muscular bed was assumed and not tested, metabolically-induced hyperemia could not be ruled out. The significance of decreased venous pressure during relaxation was expanded upon by Laughlin who hypothesized that the decrease in muscle venous pressure in the smallest veins may even be negative, creating an even larger pressure gradient and increase in flow during muscle relaxation (74).

Therefore, increases in muscle blood flow resulting from the mechanical effects of muscle contraction is implied from venous pressure decreases in the period between contractions. However, a vascular waterfall or critical pressure has been described at the arteriolar level (109), and it persists even during maximal vasodilation (140). This implies that venous pressure can only be considered the downstream pressure for inflow in muscle when the critical pressure is exceeded. If

venous pressure is below the critical pressure, the latter becomes the effective downstream pressure. This became evident in the work of Naamani and associates who examined whether muscle contractions can increase muscle blood flow independently from metabolic factors in the isolated left diaphragm and gastrocnemius of anesthetized dogs (102). Arterial inflow to muscle was not increased during the relaxation phase between muscle contractions despite a decrease in the pressure in the compliant region, measured by the venous plateau pressure during a double occlusion procedure. This is consistent with a vascular waterfall or critical pressure proximal to the compliant region in muscle. Since arterial resistance and pressure were unchanged, arterial inflow was dependent on this critical pressure. A drop in this pressure occurred during spontaneous diaphragmatic contractions which resulted in a 20% increase in phrenic arterial flow. As well, the compliance of the venous system in muscle is low (83) which suggests only a small volume is present to be discharged by contractions. They concluded that skeletal muscle contractions produce only a modest increase in muscle blood flow and the major effect of compression of the compliant region during muscle contractions is to counteract venous distension due to gravity and facilitate venous return.

Sheriff and colleagues attributed the rapid rise in total vascular conductance at the onset of dynamic exercise in

conscious dogs to the muscle pump (137). A similar rise in total vascular conductance occurred even when cardiac output was prevented from rising by atrial-ventricular linked pacing of the heart or during autonomic blockade. This rise is greater when muscle contraction frequency is increased by increasing treadmill speed. The rise in total vascular conductance at exercise onset followed the same time course as increases in central venous pressure and cardiac output and the immediate rise was unaltered by autonomic blockade. This is consistent with previously cited work indicating that the mechanical effect of muscle contractions may play a role in the rapid rise in venous return at exercise onset. The authors interpret these findings and the exercise-induced decrease in arterial pressure as fitting with Laughlin's hypothesis that muscle blood flow is increased by the muscle pump such that activation of this pump at exercise onset quickly withdraws a volume of blood from the arterial system and results in a decrease in arterial pressure. However, the authors failed to question the decrease in arterial pressure during exercise in these animals. Resting mean arterial pressure appeared to be elevated in these animals, at about 115 mmHg, perhaps due to anticipatory stress for it decreased to more normal levels during exercise. Therefore, increased muscle blood flow as a result of the muscle pump is not the only possible conclusion.

The final proposed function of the muscle pump which also stems from the observed decrease in venous pressure in muscle

following contraction, is a decrease in capillary pressure which reduces fluid filtration during exercise. This was examined in exercising humans by Stick et.al. (153) who found that extravascular volume decreased, when measured by volume plethysmography which is filtered to remove motion artifact, during cycling exercise. Extravascular volume changes were distinguished from intravascular changes by the rate of the volume response. The authors suggested their result was due to the decreased venous pressure due to the muscle pump but that the contraction-induced increase in interstitial pressure and enhanced lymph flow may also have contributed.

In summary, whereas the pumping action of muscle contractions seems to have a limited role in exercise hyperemia, it plays an important role in the transfer of blood from dependent regions to the central circulation, thereby facilitating venous return particularly at exercise onset when reflex mechanisms are not yet activated. It may also lower capillary pressures and limit fluid filtration. Additional effects on venous mechanics which may further affect venous return are unknown.

Summary

Cardiac output is altered by peripheral vascular factors which affect venous return and by changes in cardiac function. The evidence for this was reviewed in section A of this introduction. Dynamic exercise provides a means by which the integrated response of all these factors occurs in a

controlled fashion, such that increases in cardiac output are matched precisely to the metabolic requirement. How this is achieved in the context of peripheral vascular vs central cardiac responses and limits is not known. The proposed limits to maximal oxygen transport and utilization during whole body exercise were addressed in section B of this introduction. The preceding section discussed control of the cardiovascular response to exercise by neural, humoral and mechanical signals. Much of this work focused on arterial pressure and heart rate control rather than cardiac output. As a result, peripheral vascular factors were rarely considered.

The goal of this thesis is to examine the relative importance of peripheral vascular and central cardiac factors in the control of cardiac output and their interaction during the integrated response to exercise stress.

The final section of the introduction will discuss the rationale for the experiments discussed in chapters 2-6.

D. RATIONALE FOR THESIS EXPERIMENTS

Neural, humoral and mechanical factors which affect central cardiac function and alterations in the peripheral vasculature must produce the precise matching of cardiac output to metabolic demand during exercise. The studies which make up this thesis used various animal models and pathological human conditions to examine neurohumoral interactions, to separate cardiac and peripheral neural effects, to assess the effect of

altered metabolic rate during exercise and to separate mechanical and reflex neural effects on the factors which determine venous return and cardiac output.

The study described in Chapter 2 focused on neural vs local regulation of humoral vasoconstrictors endothelin-1 and neuropeptide Y. The factors controlling release of these relatively recently discovered peptides and their role in the redistribution of cardiac output is still not clear. The studies in Chapters 3 and 4 were designed to examine the interaction and limits of peripheral vascular and cardiac factors and the role of the muscle pump at exercise onset in exercising humans with and without cardiac neural input. The study in Chapter 5 addressed whether large changes in body mass would affect cardiovascular control and oxygen consumption during exercise. The study described in Chapter 6 assessed the influence of mechanical vs reflex effects of muscle contractions in different postures on the determinants of venous return and cardiac output in anesthetized dogs.

Neural Control of Endothelin-1 and Neuropeptide Y

The carotid sinus baroreflex has been implicated in the control of plasma levels of the potent vasoconstrictors endothelin-1 and neuropeptide Y (26,68,152) in addition to influencing the cardiac output response during dynamic exercise (123). These peptides are thought to play a role in circulatory control, particularly during sympathetic

activation and this potential neurohumoral interaction may impact on peripheral vascular and cardiac factors controlling cardiac output. In Chapter 2 of this thesis, I tested the hypothesis that altering carotid sinus input without changing systemic pressures would alter both plasma endothelin-1 and neuropeptide Y, whereas inducing systemic hypotension with normal carotid sinus pressure would not. Neural and local stimuli were separated in an anesthetized dog model with isolated carotid sinuses and an arterial reservoir. This allowed me to independently manipulate pressure in the carotid sinus and systemic arterial pressure. Animals were vagotomized in one series to eliminate the influence of cardiopulmonary and aortic baroreceptors and intact in another to assess the combined baroreceptor effect. Hemodynamic responses were also measured in all conditions.

Interaction of Heart and Periphery During Exercise: Heart Transplant Model

The reduced exercise tolerance of heart transplant recipients has been attributed to decreased cardiac output secondary to denervation of the heart (64,154), although animal denervation studies show no alteration of maximal work capacity (31,32). Heart transplant patients are able to increase their cardiac output during exercise stress by use of the Frank-Starling mechanism in the absence of cardiac neural innervation. Chapter 3 of the thesis examines the cardiac

output/right atrial pressure relationship of these patients compared with normal cardiac innervated subjects. Since right atrial pressure represents the common variable with respect to the heart (i.e. inflow pressure) and the peripheral vasculature (i.e. outflow pressure), we hypothesized that an increase in right atrial pressure relative to cardiac output or a plateau in this relationship during graded exercise would indicate a cardiac limit, whereas the reverse would imply a non-cardiac limit to exercise. We also compared responses in a subgroup of patients during exercise with and without stimulation of the denervated heart with a positive inotropic drug to test for a cardiac limit to exercise. Heart transplant patients were studied with a right heart catheter in place and normal subjects had an anticubital venous catheter advanced to a position outside the right atrium during the exercise studies. Heart transplant recipients provide a human model for separating cardiac and peripheral vascular neural effects on cardiac output.

The mechanical effect of muscle contraction is thought to augment venous return at exercise onset by shifting peripheral blood volume centrally (74,102,139). This has been manifested by an increase in central venous pressure at exercise onset (139). However, increases in cardiac function can also influence central venous pressure. Therefore, Chapter 4 of this thesis studied changes in central venous pressure at exercise onset and after 3 minutes of exercise in heart

transplant recipients and normal subjects to assess the effect of the muscle pump compared with neurally-mediated changes in cardiac function. In this context, heart transplant patients provide a model for isolating peripheral changes and their influence on central venous pressure from neurally mediated changes in cardiac function at the onset of exercise.

Altered Oxygen Consumption and Cardiovascular Response During Exercise: Obese Model

The concept that cardiac output increases in proportion to the absolute work rate whereas heart rate is matched to the relative work rate during dynamic exercise originated in studies in which responses of large and small muscle groups were compared in trained and untrained subjects (17,76). Another way to test this hypothesis is in a group of patients who have had large changes in body mass. Body size determines both resting and maximal oxygen consumption (158). Although absolute maximal oxygen consumption is normal in obese subjects, it is achieved at a lower work rate. Maximal oxygen consumption per kilogram of body weight is reduced in obese individuals (23,131,132) and would be expected to improve with weight loss. In Chapter 5 of this thesis, I assessed whether large changes in human body mass, induced by isolated gastric bypass surgery, alters aerobic capacity, external work capacity and the heart rate/oxygen consumption relationship during exercise. This patient group provided a model for

studying this relationship when oxygen consumption at a given level of work was markedly different, due solely to body mass changes. The hypotheses were that heart rate would follow relative oxygen consumption; peak absolute oxygen consumption would not change if activity level was the same, but oxygen consumption per kilogram would increase markedly due to the large weight reduction. We also addressed a clinical question which was whether a failure to lose weight following surgery is due to a reduced normalized energy expenditure. Responses of four groups were compared: preoperative obese patients; postoperative obese patients who failed to lose weight; postoperative obese patients who normalized their body weight; control non-obese subjects. As well, preoperative obese patients were assessed longitudinally at 6 months after surgery.

Control of Venous Return during Exercise: Mechanical vs Reflex Effect of Muscle Contractions

Venous capacitance is decreased when adrenergic drugs are administered, although changes in venous resistance are more variable (10,99). Carotid sinus hypotension decreases venous capacitance and venous resistance in the splanchnic region and leads to a redistribution of blood flow (26). A substantial blood flow redistribution also occurs during dynamic exercise. Similar changes in venous capacitance and resistance would account for the large increases in cardiac output which occur

at near maximal exercise intensities (46,84). The study described in Chapter 6 assesses the effects of electrically stimulated dynamic muscle contractions on venous resistance, capacitance and compliance. I hypothesized that the simulated exercise would result in overall 1) decreases in venous capacitance, which could result from both reflex adjustments and recruitment of volume during muscle contractions. 2) decreases in venous resistance which could also be due to both reflex effects and muscle contractions. 3) lack of change or a small decrease in venous compliance due to muscle contraction. To isolate the mechanical pumping effect of muscle contractions on venous mechanics, reflex adjustments mediated by muscle afferents were blocked by cold blockade proximal to the stimulating electrodes. I hypothesized that the muscle pump would contribute to decreases in venous capacitance and compliance, although the effect may be small compared with reflex changes that result in sympathetic activation. Measurements were made in anesthetized dogs during intermittent bilateral sciatic nerve stimulation both in the supine and leg-dependent position in order to ensure filling of hindlimb veins.

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CHAPTER 2

EFFECT OF BARORECEPTOR ACTIVATION AND SYSTEMIC HYPOTENSION ON PLASMA ENDOTHELIN-1 AND NPY

Chapter Link

Potent vasoconstrictors endothelin-1 and neuropeptide Y are thought to play a role in circulatory control, particularly during sympathetic activation. The carotid sinus baroreflex has been implicated in the control of these peptide levels in plasma in addition to influencing the cardiac output response during dynamic exercise. The study described in this chapter examines the independent effect of carotid sinus pressure and systemic arterial pressure on plasma levels of endothelin-1 and neuropeptide Y. Such neurohumoral interactions may impact on peripheral vascular factors which control cardiac output such as blood flow distribution and regional venous capacitance.

A. ABSTRACT

To determine whether endothelin (ET-1) and neuropeptide Y (NPY) release are controlled by the carotid sinus (CS) baroreceptor or local endothelial mechanisms, we isolated and pump-perfused the CS in 8 chloralose anesthetized dogs and controlled systemic arterial pressure (SAP) with an elevated reservoir connected to both femoral arteries. This allowed the SAP to be kept constant while CS pressure was varied from 55.8±2.0 (low CS) to 192±1.9 mmHg (high CS) or CS pressure to be kept constant while SAP was lowered to 53.9±1.8 mmHg (low SAP). There was no significant change in ET-1 when CS pressure was varied (control=2.08±0.50; low CS=2.18±0.51; high CS=2.11±0.38 pg/ml) but ET-1 was significantly higher during low SAP (2.93±0.49, $p<.05$). This increase was not observed with vagi and CS intact in 6 dogs or with vagi intact and CS constant in 4 dogs. In contrast, plasma NPY was significantly higher in the low CS (619.13±66.87) vs high CS condition (528.88±45.19 pg/ml, $p<.05$) and did not change during hypotension. In conclusion, NPY, but not ET-1, is affected by CS baroreceptor manipulation, and plasma ET-1 increases in response to hemorrhagic hypotension when modulating reflexes are abolished.

B. INTRODUCTION

Endogenous vasocontrictors, such as endothelin-1 and neuropeptide Y are among the new peptides thought to play a role in the control of circulation. Endothelin-1 is the most potent vasoconstrictor peptide yet identified and is found in the plasma of normal animals and humans (2,36). It is synthesized by endothelial cells as well as by the paraventricular nucleus of the hypothalamus (29,37). In vitro studies indicate that it is released from endothelial cells in response to various mechanical and chemical stimuli (16,17,33,38) and acts as a modulator of vascular tone.

The control mechanisms for the release of endothelin-1 have not been fully elucidated. In particular, it is not known whether the arterial baroreflex affects endothelin release. Binding sites have been demonstrated in vascular smooth muscle of the aorta and pulmonary artery, the heart, kidney and brain, particularly in areas concerned with the regulation of arterial pressure (11). Endothelin-1 concentration is elevated in hypotensive states such as congestive heart failure in dogs (5), endotoxic shock in rats, pigs and dogs (20,24,33), and cardiogenic shock in humans (6). Increased concentrations have also been observed in response to a 60 degree head-up tilt in normal human subjects, which suggests that the efferent limb of the baroreceptor reflex may play a role in the release of endothelin-1 (10,32). These authors have speculated that endothelin-1 (ET-1) may act as a neuropeptide which is

released from nerve terminals directly or from the endothelium indirectly in response to decreased carotid sinus stimulation. While it has not been shown that plasma ET-1 levels increase as a direct result of changes in sympathetic outflow, the latter is undoubtedly increased in hypotensive disorders. Alternatively, the increased concentration during hypotension could result from the release of ET-1 from endothelial cells through local mechanisms (i.e. decreased shear stress or local hypoxia). This question of neural versus local control of endothelin-1 release during hypotension led to the present study.

The 36 amino acid peptide neuropeptide Y (NPY) is a potent vasoconstrictor which is found in the brain, adrenal medulla and peripheral sympathetic nerves where it is co-stored with norepinephrine. It is thought to play a role in the sympathetic control of vascular tone (12). Elevated plasma levels of NPY have been found during sympathetic activation in healthy subjects during dynamic exercise and cold pressor test (13,19). However, in contrast to catecholamines, no increase in circulating NPY was found after 2.5 minutes of head-up tilt in humans (19). This is because NPY is released over a longer time course than catecholamines in response to sympathetic activation (25). Release of NPY in response to carotid sinus activation has not been directly examined.

The purpose of this investigation was to determine whether the release of endothelin-1 and NPY are regulated through a

neural reflex mechanism mediated by the carotid sinus baroreceptor. We tested this in a preparation in which we independently manipulated carotid sinus pressure (neural stimulus) and systemic arterial pressure (local stimulus). We also examined the effect of an intact vagus nerve on plasma endothelin-1 levels during hypotension, in order to assess the role of the aortic and cardiopulmonary baroreceptors in the regulation of endothelin-1 release under these conditions.

C. METHODS

Surgical Preparation

Twenty-one mongrel dogs of either sex weighing 21.4-32.7 kg (Mean=28.3±S.D.=3.43) were studied. They were anesthetized with 25 mg/kg thiopental sodium followed by 80 mg/kg α-chloralose which was supplemented as necessary. The animals were intubated, connected to a volume respirator (Harvard Apparatus,South Natick,MA)and ventilated with room air mixed with 30% oxygen. A schematic representation of the preparation is shown in Figure 2.1. A midline incision was made in the neck and a catheter was placed in the left carotid artery to monitor arterial pressure (P_a) and obtain blood samples during the experimental protocol. Fluids were administered through a catheter placed in the left external jugular vein. We isolated the carotid sinuses by ligating the internal and external carotid arteries and main branches distal to both sinuses and cannulating the common carotid artery proximal to the right

carotid sinus. The two cannulas, joined by a Y-connector, were inserted distally in order to perfuse the carotid sinuses via a pump (Masterflex®,Cole-Parmer Instrument Co.,Chicago,Il.) connected to the proximal end of the right carotid artery. Flow in the isolated carotid sinus was pulsatile. Intact function of the carotid sinus was tested by increasing the perfusion pressure and observing an appropriate drop in arterial pressure and heart rate. This was done in order to ensure the integrity of the CS preparation. Carotid sinus pressure (P_{cs}) was measured from a side-port in the tubing distal to the pump. The carotid sinus pressure was set at approximately 200 or 50 mmHg by altering the speed and direction of the pump perfusing the sinuses. Sodium heparin (10,000 USP, i.v.) was administered prior to connecting the animal to the pump. Both femoral arteries were cannulated and connected by tubing to an elevated external reservoir in order to maintain a constant arterial pressure during carotid sinus manipulations. The reservoir was primed with 500 ml of dextran and could be lowered in order to induce hypotension. This preparation allowed us to independently control carotid sinus and arterial pressure. Bilateral cervical vagotomy was performed in some animals, in order to eliminate the effects of cardiopulmonary and aortic baroreceptors. We are confident that the vagotomy was effective since we cut both vagus nerves at the cervical level, which, in the dog, is above the sympathetic trunks. The few aortic baroreceptor fibres which

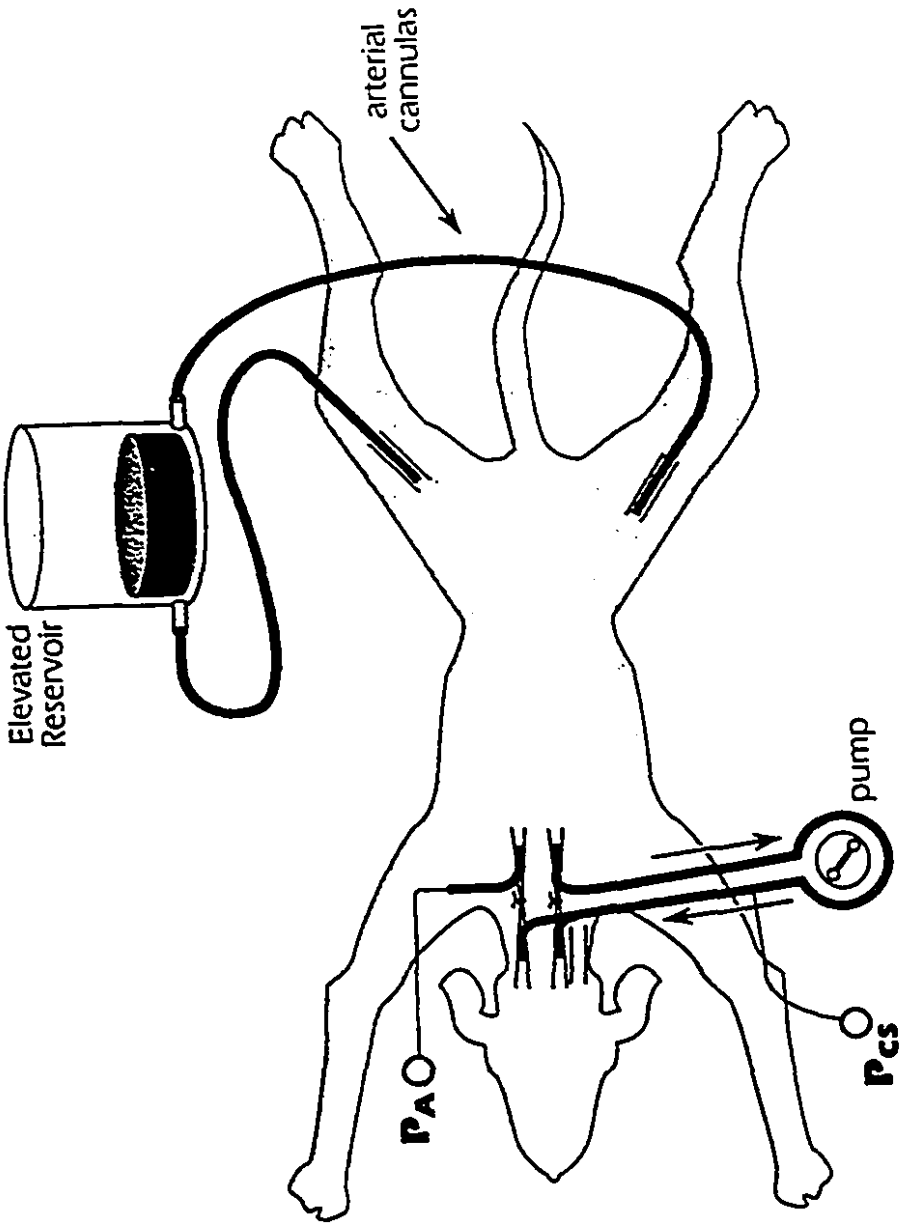

FIGURE 2.1. Experimental preparation. Pa, arterial pressure; Pcs, carotid sinus pressure. See text for details.

do not join the cervical aortic nerves but instead traverse the vagal nerves are of questionable functional significance (1).

A Swan-Ganz catheter was inserted via the jugular vein into the pulmonary artery in order to measure cardiac output by the thermodilution method and monitor central venous pressure (CVP) and pulmonary wedge pressure (P_w).

Measurements

An 18 gauge needle was inserted in the tubing at the base of the external reservoir to measure pressure in order to estimate volume changes in the reservoir. Calibration with known volumes was made at the end of the experiment. Pressures were measured with Trantec transducers (American Edwards Laboratory, Irvine CA). The signals were amplified with a preamplifier (Hewlett-Packard 8805A) and the outputs were recorded on an eight-channel recorder (Hewlett-Packard 7418A). Blood samples were collected in chilled glass tubes containing EDTA and were centrifuged at 3000 rpm for 15 minutes at 4°C. Plasma was separated and frozen at -20°C until assay. Cardiac output was measured by thermodilution with a cardiac output computer (Abbott Critical Care Systems 3300, Abbott Labs, North Chicago,IL). Measurements were made in triplicate to obtain values in agreement by ±10%. Values are normalized to body weight and expressed as ml/min/kg. The animals' metabolic status was monitored by analysis of expired gases using a comercial unit (Medgraphics™ Critical Care Monitor, Medical

Graphics Corp.,Minniapolis,MN) which was connected via a pneumotachometer to the endotracheal tube. This provided a measure of whole body oxygen consumption which was used with the measured arterial-mixed venous oxygen content difference to calculate cardiac output by means of the Fick equation. This was done in some animals in order to confirm thermodilution values.

Experimental Protocols

Protocol 1: Eight dogs were prepared as described above and the vagi were cut. After a 45 minute stabilization period, control measures were made and three 15 ml blood samples were withdrawn from the carotid artery at five minute intervals. Three randomly assigned interventions were then made in each dog: 1) high carotid pressure condition in which the carotid sinus pressure was abruptly increased to approximately 200 mmHg by increasing the output of the pump, while arterial pressure was maintained constant at the control value by allowing volume shifts from the reservoir to the dog; 2) low carotid condition where the carotid sinus pressure was rapidly decreased to 50 mmHg while the arterial pressure was maintained at control levels by allowing a volume shift to the reservoir; 3) systemic hypotension condition, which was produced by abruptly lowering the height of the reservoir, which caused arterial pressure to drop from control levels to approximately 50 mmHg while carotid sinus pressure was kept at control levels. Half of the animals were exposed to the

carotid sinus manipulation first and the other half received the hypotension stimulus first. We sampled 15 ml of arterial blood at 5, 10 and 15 minute intervals following the changes in pressure in each condition. These time intervals were selected in order to match the human tilt protocol of Stewart et.al. (32)

Blood was also sampled before the surgical preparation to assess the effect of surgical stress on plasma endothelin-1 and NPY concentrations. In four animals, possible effects of anesthesia on plasma endothelin-1 levels were studied by sampling blood before and after α-chloralose anesthesia was administered. After the preparation was allowed to stabilize for 45 minutes, we began the experimental protocol.

We measured cardiac output by thermodilution just prior to blood sampling in each condition. In five animals. cardiac output was also calculated from the measured oxygen consumption and arterial-mixed venous oxygen content difference using the Fick equation.

Protocol 2: In order to examine the effect of vagally mediated aortic and cardiopulmonary baroreceptors on plasma endothelin-1, during the hypotension condition, six dogs were studied in a similar manner to protocol 1, but the carotid sinus was not isolated and the vagi were intact. Thus the only manipulation was the change in blood volume produced by adjusting the reservoir.

The protocol was repeated in four dogs with carotid sinus

pressure maintained constant as in protocol 1 and vagi intact.

Measurement of Plasma Endothelin-1

Plasma endothelin-1 was determined by a modification of the previously described radioimmunoassay (6). Samples were extracted with SepPak C18 cartridges (Walters, Mississauga, Ontario, Canada) activated with methanol, 8 mol/l urea and water, and eluted with methanol (recovery= 75±3%). Samples and standards (endothelin-1, Peninsula Laboratories, Belmont CA.) were reconstituted in assay buffer and incubated for 24 hours with anti-endothelin-1 antibody (Peninsula Laboratories) at 4°C. The addition of approximately 4,000 cpm of [^{125}I]endothelin-1 (Peninsula Laboratories) was followed by a second 24-hour incubation. Bound radioactivity was separated from free radioactivity by the second antibody method and evaluated after logit/log transformation. Immunoreactive endothelin-1 (irET-1) is presented after correction for recovery.

The limit of detection, defined as the least amount of irET-1 distinguishable from zero at a 95% confidence level, was 0.2 pg/ml of blood.

Measurement of Plasma NPY

Immunoreactive NPY in plasma was determined by a radioimmunoassay adapted from the method of Peninsula Laboratories (Belmont, CA). Extraction was accomplished by adding 1 ml of hydrochloric acid-ethanol mixture to each 500 µl of plasma. After agitation for 30 seconds, the samples were

centrifuged at 3000 cpm for 20 minutes at 4°C. The supernatants were separated and then evaporated at room temperature under nitrogen gas. After reconstitution with assay buffer the samples were incubated for 72 hours with an antibody raised against porcine NPY (Peninsula Laboratories). The addition of approximately 6,000 cpm of [^{125}I]NPY (Amersham Canada Ltd., Oakville, Ont.) was followed by a 48-hour incubation. The antibody exhibited a 64% cross-reactivity with human NPY and no cross-reactivity to other structurally related peptides, such as peptide YY or bovine pancreatic peptide. Standard curves were prepared using synthetic human NPY (Sigma Chemicals, St.Louis, MO) which only differs from porcine NPY in one of the 36 amino acids. Plasma NPY concentrations were measured in duplicate in 500 µl of plasma. The detection limit for NPY in this volume is 30 pg/ml.

Statistical Analysis

Data were analyzed by means of a two factor analysis of variance for repeated measures which tested for main effects of time and experimental intervention in the two protocols. Main effects were considered statistically significant at the 95% confidence level and post-hoc Tukey tests were applied to assess differences between means. All data are reported as mean ± standard error.

D. RESULTS

Protocol 1

An example of the experimental record is shown in Figure 2.2. This illustrates that the reservoir effectively buffered changes in arterial pressure in response to rapid increases or decreases in carotid sinus pressure. The system had a time lag of 35-45 seconds. The mean values ± standard error for carotid sinus pressure, arterial pressure and heart rate during control and the three experimental conditions are given in Table 2.1. Systemic arterial pressures were similar in control, low and high carotid sinus pressure conditions. Heart rate was significantly increased and decreased from control during low and high carotid sinus pressure conditions respectively ($p<.05$). No change in heart rate occurred during hypotension at a constant carotid sinus pressure. Oxygen consumption was stable across all conditions, with a tendency to decrease during hypotension as expected.

Plasma Endothelin-1 Levels: Analysis of variance of plasma endothelin-1 (ET-1) levels showed no effect of time but an effect of experimental intervention ($F=4.55$, $p<.025$, $df=3,21$). Since there was no time effect, we report the mean value of ET-1 measured from the 3 serial blood samples collected in each condition. There was no difference in ET-1 measured before and after α-chloralose anesthesia in 4 dogs (0.34±0.28 vs 0.64±0.20 pg/ml). Values were almost identical in 3 dogs and increased in 1 animal. Mean ET-1 values after surgery were

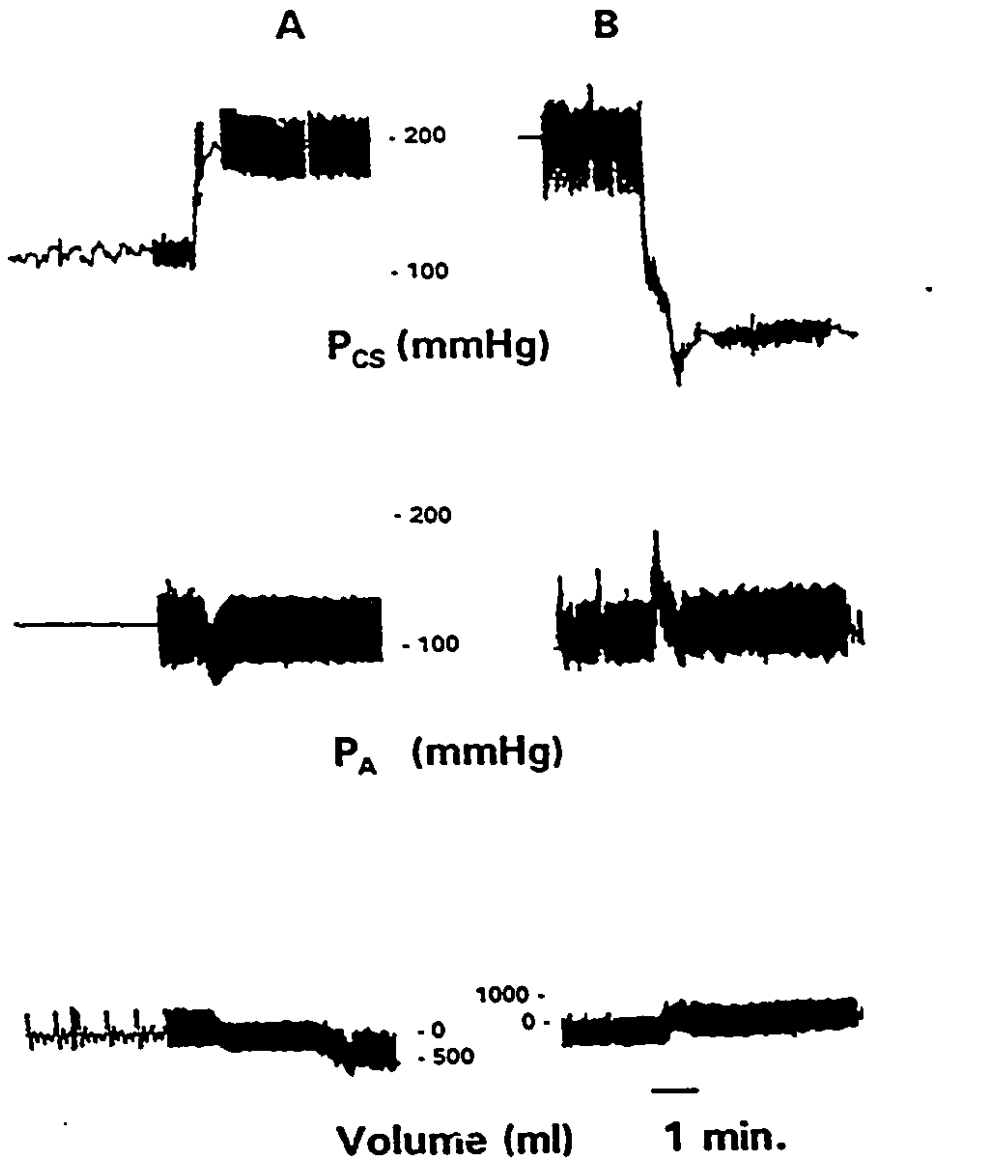

FIGURE 2.2. Sample of experimental record demonstrating time lag of 35-45 seconds in buffering arterial pressure (*middle panel*) during increases (*top panel* A) and decreases (*top panel B*) in carotid sinus pressure. Arterial pressure is normalized by a volume shift from reservoir to dog in A (*lower panel*) and a shift in volume from dog to reservoir in B.

TABLE 2.1. Protocol 1 values are presented as mean ± S.E. CSP, carotid sinus pressure; SAP, systemic arterial pressure; VO_2, oxygen consumption; HR, heart rate; a-v O_2, arterial-mixed venous oxygen content difference; CO-therm, cardiac output measured by thermal dilution; CO-fick, cardiac output measured by direct fick calculation; ET-1, endothelin-1; NPY, neuropeptide Y; *,#, values significantly different from control at p<.05 and p<.01 respectively; +, values significantly different from low CSP condition at p<.01. (N=8)

	CONTROL	LOW CSP	HIGH CSP	HYPOTENSION
CSP mmHg	121.75±2.73	55.75±2.03	191.71±1.94	121.21±2.8
SAP mmHg	117.13±2.81	118.46±3.62	108.63±6.84	53.88±1.8
HR bt/min	185.38±3.17	211.25±6.3*	151.25±5.4*	185.88±5.21
VO_2ml/min	154.6±17.14	157.0±27.26	150.6±16.98	124.8±20.5
a-v O_2ml/l	69.60±6.89	89.33±8.87*	58.83±8.24+	109.8±6.9#+
CO-therm ml/min/kg	94.99±12.51	72.33±6.29	122.6±11.7+	52.17±5.1#
CO-fick ml/min/kg	83.13±11.78	66.53±18.69	115.6±20.2+	45.8±7.18#
ET-1 pg/ml	2.09±0.50	2.18±0.51	2.11±0.38	2.93±0.49*
NPY pg/ml	590.25± 69.72	619.13± 66.87	528.88± 45.19+	580.88± 59.53

about 3-fold higher than those measured in the pre-operative sample (0.64±0.20 vs 2.08±0.50 pg/ml, $p<.025$)(Figure 2.3). Mean arterial plasma ET-1 levels did not change in response to high or low carotid sinus pressure stimulus (2.11±0.38 and 2.18±0.51 pg/ml respectively, $n=8$). However, mean ET-1 was significantly higher during systemic hypotension (2.93±0.49 pg/ml) compared with control, low and high carotid sinus pressure conditions ($p<.05$) (Figure 2.3). This increase occurred when carotid sinus pressure was maintained at normal levels in vagotomized animals, i.e. during hypotension with compensatory reflexes inoperative.

Plasma NPY Levels: Pre-operative NPY values were not significantly different from control values post-surgery (557.6±68.5 vs 590.3±69.7 pg/ml). Analysis of NPY data at 15 minutes showed an effect of experimental condition ($F=4.75$, $p<.025$, $df=3,21$), with mean plasma NPY significantly higher in the low carotid sinus pressure condition compared to high carotid sinus pressure condition ($p<.01$). Mean plasma NPY during systemic hypotension, with carotid sinus pressure maintained at control levels, was not significantly different from control. Mean data is presented in Figure 2.4.

Cardiac Output: There was an effect of experimental condition on cardiac output ($F=13.54$, $p<.001$, $df=3,21$) but no time effect. Mean values are reported in Table 2.1. Mean cardiac output was significantly higher during the high carotid condition (122.59±11.68 ml/min/kg,) than both the low

carotid sinus (72.34±6.29 ml/min/kg, p<.01) and hypotension (52.17±5.09 ml/min/kg, p<.01) conditions. Mean cardiac output was also significantly lower during hypotension than during control (52.17±5.09 vs 94.99±12.51 ml/min/kg, p<.01), reflecting volume changes in the reservoir. There was no correlation between changes in cardiac output and changes in ET-1. There was good agreement between values obtained using the thermodilution and Fick methods (r=0.88, n=5).

Central Pressures and Hematocrit: There was no significant change in central venous pressures, pulmonary wedge pressures or hematocrit during the first and second baseline periods.

Protocol 2

Mean arterial pressure was decreased from 122.8±4.4 mmHg to 63.1±2.5 mmHg for the hypotensive condition by lowering the reservoir. Carotid sinuses and vagi were left intact. In contrast to protocol 1, we found no change in mean ET-1 levels during hypotension (0.57±0.09 pg/ml), compared with control (0.51±0.04). There was also no change in plasma ET-1 during hypotension in four additional dogs with isolated carotid sinus and pressure maintained constant (0.83±0.32 vs 1.07±0.69 pg/ml). Of note, baseline ET-1 levels were considerably lower than in protocol 1. As in protocol 1, cardiac output dropped from 128.4±37.7 to 64.3±15.8 ml/min/kg during hypotension.

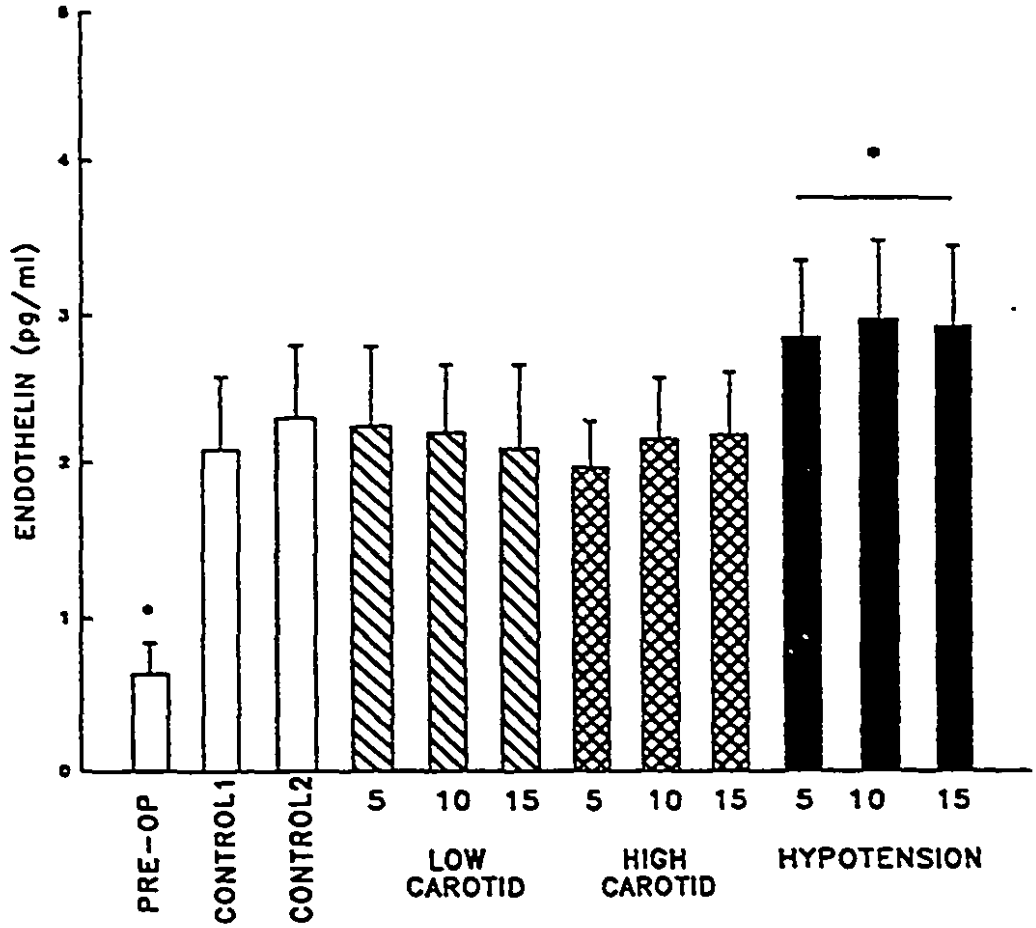

FIGURE 2.3. Protocol 1. Plasma endothelin-1 levels (Mean±S.E.) are shown during pre-operative period, 2 post-surgery baseline control periods (*open bars*) and at 5,10 and 15 minutes following each of the three experimental interventions. Low CS (*diagonal striped bars*), high CS (*cross-hatched bars*) refer to carotid sinus pressure; normal CS, hypotension (*solid bars*) refers to low systemic arterial pressure induced by hemorrhage into the reservoir with carotid sinus pressure maintained constant; *, values significantly different from control 1 at $p<.05$, n=8 vagotomized dogs.

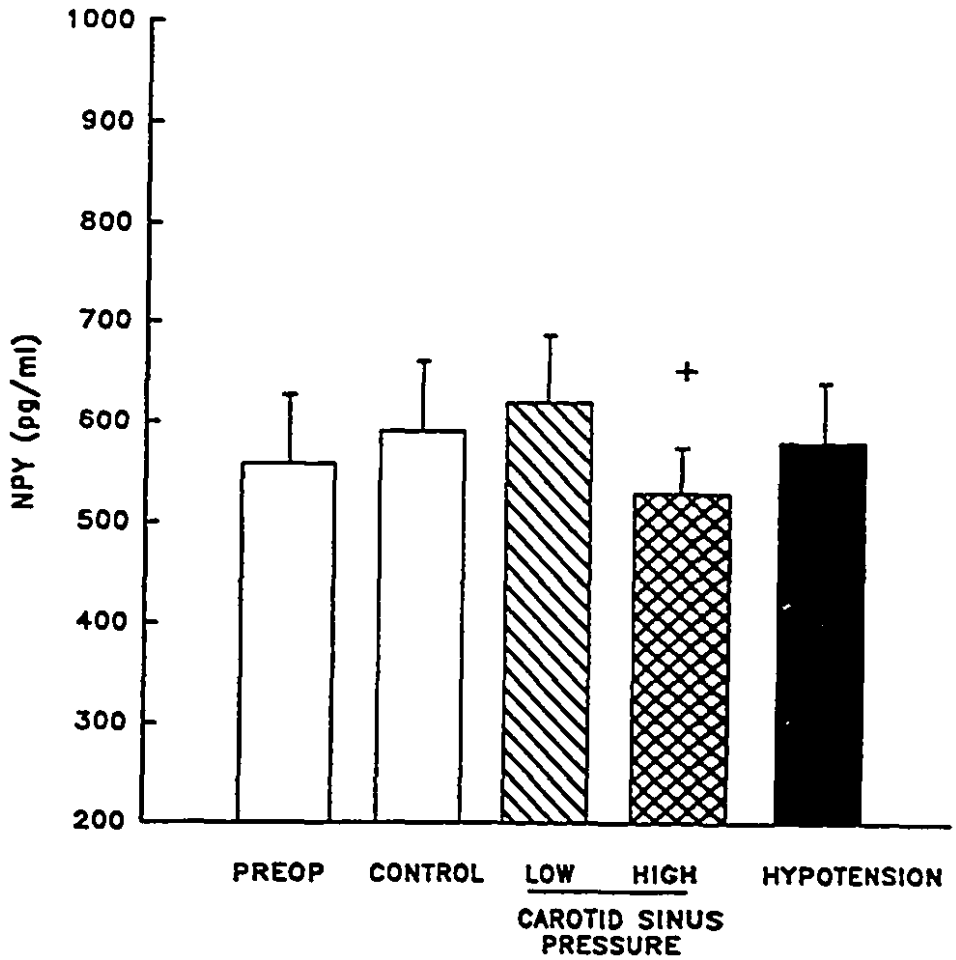

FIGURE 2.4. Protocol 1. Plasma NPY values (Mean±S.E.) before and after surgical preparation and at 15 minutes following each condition. +, value significantly different from low carotid sinus pressure (CSP) condition at $p<.01$, $n=8$.

E. DISCUSSION

We found that plasma levels of endothelin-1 increase during systemic hypotension but this increase is not mediated by the carotid sinus. The increase was not observed when the vagus nerve and carotid sinus were intact. Conversely, the release of NPY was responsive to carotid sinus pressure manipulation but not to systemic hypotension when carotid sinus pressure is constant.

Before discussing the results, a number of technical factors should be considered. There was a large variability in plasma endothelin-1 levels between animals, despite the fact that care was taken to minimize variability by maintaining standardized procedures. Of particular importance, sterilization of the lines and reservoir system was necessary to protect against endotoxic contamination, a potent stimulus for endothelin-1 release. However, it was not possible to control all factors, such as variability in the response of individual animals to anesthesia and surgical preparation. Alpha chloralose anesthesia, which was used in these experiments, does not significantly alter carotid sinus reflexes compared with depression of these reflexes seen with other agents (8).

The high control endothelin-1 levels in protocol 1 may have masked a response to carotid sinus manipulation. However, this is unlikely because an increase in endothelin-1 was observed with a magnitude of systemic hypotension similar to the low

carotid stimulus (i.e. 50 mmHg). Unfortunately, catecholamines were not measured in this study, however, an indication of sympathetic outflow in protocol 1 can be obtained by examining the heart rate response listed in Table 2.1. These responses to low and high carotid sinus pressure conditions would argue against a maximal activation of the sympathetic nervous system by the preparation alone. Of importance, we randomly assigned the carotid sinus manipulations and the hypotension (with constant carotid sinus pressure) condition, and observed that ET-1 levels returned to baseline following hypotension in 4 animals where the latter was performed first.

Endothelin-1 Release during Hypotension

When the vagi were cut and carotid sinus pressure maintained constant, plasma endothelin-1 concentrations increased almost 1.5 fold during hypotension. The levels reached are comparable to the magnitude seen in patients with chronic congestive heart failure (7,15), as well as dogs with congestive heart failure induced by ventricular pacing (5,14). The mean endothelin-1 value of 2.93 pg/ml seen during acute hypotension in protocol 1 is comparable to the value of 3.65 pg/ml observed by Cernacek and Stewart who used the same assay in patients with cardiogenic shock (6). Cavero et.al. used a similar assay procedure and also observed a mean arterial endothelin-1 level of 3.25 pg/ml in anesthetized dogs with heart failure (5).

Endothelin levels did not change with changes in the

carotid sinus pressure. Thus, factors related to hemorrhage must explain the increase in endothelin during hypotension, There are a number of possibilities. 1) Decreases in intimal shear stress could have increased the release of endothelin from endothelial cells, as has been seen in cultured endothelial cells (17,38) and intact arteries (16). 2) Hypoperfusion during the hemorrhagic hypotension may have interfered with clearance mechanisms through the lung and kidney and resulted in elevated plasma levels. 3) Local hypoxia from hypoperfusion may have stimulated endothelin release under these conditions as has been shown in cultured endothelial cells (28). 4) Hypotension may have resulted in activation of peripheral sympathetic afferent activity, which can release other compensatory peptides (21). 5) Finally, a decrease in cerebral perfusion could have resulted in a central release of endothelin from the posterior pituitary, which can not be distinguished from peripheral release by our sampling methods.

We were surprised to find that plasma endothelin-1 levels did not increase when hypotension was induced by hemorrhage and the carotid sinus function and vagi were intact. The similarity of the values before and after the hypotension make it unlikely that this was a type II error. Pernow et.al. also reported that plasma endothelin did not increase in pigs, with intact vagi, when mean arterial pressure was lowered by a hemorrhage to the same degree as in our study (24). The most

likely explanation is that the response we observed during hypotension with the vagi cut was masked by the complex interaction of neural and humeral factors when both vagus and carotid sinus or vagus only were intact. This could suggest an inhibitory influence of the vagus, either direct or indirect, on endothelin-1 release under normotensive conditions and may explain the increased control levels seen in protocol 1. Vagally mediated cardiac and aortic baroreceptors are known to play a major inhibitory role in the regulation of other vasoactive peptides, particularly arginine vasopressin and renin (3,27). Indeed a parodoxical vagal activation can occur when strong sympathetic activation is combined with low blood volume as has been noted by Oberg and Thoren, (22). We cannot separate the influence of aortic and cardiopulmonary baroreceptors or cardiac vagal afferents in this preparation.

Accordingly, the small transient increases in endothelin-1 plasma levels with upright tilt in awake humans (10,32) may be explained by 2 possibilities: 1) an unloading of atrial stretch receptors with postural change which resulted in a decrease in vagal tone, or 2) altered local mechanical and chemical factors induced by changes in blood flow during tilt. This human data differs from our result in anesthetized dogs with all reflexes intact and may reflect a difference in volume load (31).

NPY release during carotid sinus stimulation

Plasma NPY levels were higher during the low carotid sinus

pressure condition than the high carotid condition, indicating that NPY release is controlled at least to some extent by the carotid baroreceptor. This agrees with reports that NPY is co-released with norepinephrine from peripheral sympathetic nerve terminals during strong sympathetic activation (4,25,26), or high frequency nerve stimulation (23). In humans, mild levels of sympathetic stimulation such as isometric handgrip exercise, tilting and mild dynamic exercise do not increase plasma NPY levels (19,25,35). However, more intense activation, such as heavy dynamic exercise, increases plasma levels of NPY from 67% to 400% (13,19,25,35). In animal studies the release of NPY and norepinephrine from peripheral noradrenergic nerve endings is not always linked, suggesting that a threshold level of sympathetic stimulation is required for co-release (26,34).

The increase in plasma NPY during low carotid sinus pressure conditions was small. This may reflect a more moderate change in sympathetic activation due to elevated baseline levels as a result of surgical stress. Alternatively, it could be the result of an adaptation of the carotid sinus baroreceptor over the 15 minute period of stimulation (30), significantly reducing the magnitude of sympathetic stimulation. Catecholamine measurements would have been an asset in this regard, however, as mentioned above, release of norepinephrine and NPY are not always linked. An increased clearance of NPY could explain the decrease in plasma levels

with high carotid sinus pressure, however it is unlikely that proteases which degrade NPY would be altered in this condition.

Finally, although hemorrhage is a potent stimulus for NPY release (4,18), our results demonstrate that this effect is completely prevented when neural inputs are either controlled (i.e. carotid sinus) or eliminated (i.e. aortic and cardiopulmonary baroreceptors).

Hemodynamics

Cardiac output was significantly lower in the low vs high carotid sinus pressure condition in protocol 1. This can be explained by a decrease in total venous capacitance, primarily due to decreases in unstressed volume and venous resistance in the splanchnic region during carotid sinus hypotension (9). In an intact circulation, this would lead to an increase in vascular stressed volume and an increase in venous return and cardiac output. However, in the present study the arterial reservoir added another compliant region outside the circulation. Therefore, the transient rise in arterial pressure, due to the increased peripheral resistance, caused blood to leave the dogs' circulation and move into the reservoir. This resulted in an effective loss of stressed volume. The combination of loss of stressed volume and normalization of arterial pressure more than accounted for the changes in capacitance and flow redistribution induced by carotid sinus hypotension, and resulted in a decrease in

venous return and cardiac output.

Conclusion

We found no evidence for release of endothelin-1 through a neural mechanism involving the carotid sinus baroreceptor, whereas, neuropeptide Y is affected by carotid sinus pressure. Hemorrhagic hypotension with the vagus cut and carotid sinus pressure constant resulted in an increase in endothelin-1 in plasma.

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CHAPTER 3

Cardiac vs Non-cardiac Limits to Exercise Following Heart Transplantation

Chapter Link

In the previous chapter I addressed neurohumoral interactions which may influence vascular tone and blood flow distribution. Redistribution of blood flow is a determinant of venous return and contributes to its increase during dynamic exercise. The next paper focuses on cardiac factors in cardiac output control, specifically, whether cardiac function limits exercise capacity. If so, it would be especially evident in heart transplant patients since their heart is denervated and therefore reliant on circulating catecholamines to increase heart rate and contractility. These patients are also known to have a decreased exercise tolerance compared with age-matched normal subjects. Thus, heart transplant patients provide a model for studying the relative importance of peripheral vs cardiac factors in cardiac output control during exercise since peripheral and cardiac neural effects can be separated.

A. ABSTRACT

To determine whether the reduced exercise capacity of patients after heart transplantation (HT) is primarily a result of decreased cardiac or peripheral vascular factors, we examined the cardiac output (CO) and right atrial pressure (Pra) relationship during graded cycle ergometry. We studied 12 male patients (Mean age 51.2± S.D. 15.3) 35.3± 12.5 weeks following HT and 6 young healthy males. Patients had a normal increase in cardiac output with increasing oxygen uptake ($\dot{V}O2$) (CO =0.00597$\dot{V}O2$ + 4.4, r=.83). Mean (±S.E.) heart rate increased from 97.0± 5.0 at rest to 146.9± 6.9 bt/min at peak effort, compared with the increase of 67.2± 1.9 to 187.2± 2.5 bt/min in normals. Pra in patients increased from 1.6± 1.0 at rest to 8.9± 1.6 mmHg during mild exercise but did not increase at higher work rates (12.6± 1.8 and 13.5± 1.6 mmHg) even though cardiac output continued to increase. In normals, after an initial increase in the rest to exercise transition, there was little change in Pra with increasing cardiac output. Aerobic capacity (peak $\dot{V}O_2$) did not increase when cardiac function was increased with dobutamine during exercise in 2 patients. The steep increase in cardiac output relative to Pra during severe exercise in HT patients argues against the heart as the sole limiting factor during maximal effort.

B. INTRODUCTION

Heart transplant patients can exercise to 50-70% of the capacity of normal individuals of the same age (2,8,23,35,42). The actual level achieved is influenced by how long after surgery patients are evaluated, their level of physical activity and their drug therapy (4,26,27,40,41). Both cardiac and peripheral factors could explain the reduced capacity in these patients. Decreased cardiac adaptation to exercise may occur from the denervation (40,42,43). Alternatively, a decrease in ventricular compliance could impair ventricular filling (22,24,33,39). Peripheral factors could include decreased skeletal muscle function, impaired vasodilation and deconditioning. Persistent impaired skeletal muscle function has been reported in heart transplant patients and is thought to be due to pre-transplant congestive heart failure (30,45). An impaired vasodilatory reserve has also been suggested (29,31,46). Both these abnormalities may also result from deconditioning (5,29) and exercise training has been shown to improve exercise tolerance as well as metabolic function of muscle (27,44).

Our aim in this study was to determine whether the reduced exercise capacity of patients after heart transplantation is more dependent on cardiac or non-cardiac factors. In order to distinguish between cardiac and peripheral vascular limits to exercise, we examined the cardiac output/right atrial pressure relationship during graded exercise to a maximal effort. Since

right atrial pressure is the outflow pressure for the peripheral circulation as well as a measure of the preload of the heart (17,19), any mismatch between peripheral circuit factors and cardiac response would be reflected by changes in right atrial pressure. This can be explained as follows.

The normal heart adjusts to increases in venous return during exercise in primarily two ways. One is through the Frank-Starling mechanism by which a rise in right atrial pressure is associated with a rise in cardiac output, expressed graphically by Guyton (17) as movement up or down a cardiac response curve (open circles in Figure 3.1). The other way is through a neuro-humorally mediated improvement in pump function through an increase in heart rate and/or contractility. In the denervated heart this occurs in response to circulating catecholamines. When cardiac output increases with an improvement in pump function, steady state cardiac output increases with a decrease or no change in right atrial pressure and is graphically represented by a shift in the cardiac function curve (closed circles in Figure 3.1).

The interaction of cardiac response and venous return can be examined with cardiac response and venous return curves on the same axes (17) as seen in Figure 3.1. A shift in fractional blood flow distribution from slow to fast time constant beds such as from the splanchnic area to working muscle, and/or a decrease in venous resistance increase the negative slope of the venous return curve, and a decrease in

venous capacitance and/or venous compliance shifts the venous return curve to the right (9,16) (Figure 3.1). We refer to this as an increase in circuit function. The intersection point of the cardiac response and venous return curves is the steady state cardiac output and venous return. The right atrial pressure at which this occurs thus reflects the balance of changes in central or "cardiac" and peripheral vascular factors (17).

Studies in animals and humans indicate that central and peripheral changes are balanced in normal individuals during upright dynamic exercise, since increases in cardiac output occur with little change in right atrial pressure (3,18,21,36). We consider, therefore, that a steady state increase in cardiac output without an increase in right atrial pressure during exercise implies that the improvement in the cardiac response matches the changes in circuit factors (closed circles in Figure 3.1). On the other hand, little further increase in cardiac output with continuing increases in right atrial pressure at peak exercise in heart transplant patients would provide evidence for a central or cardiac limit to exercise (open circles in Figure 3.1). We therefore examined the cardiac output/right atrial pressure relationship in 12 heart transplant patients and 6 normal subjects to determine whether cardiac or peripheral factors predominate to limit exercise.

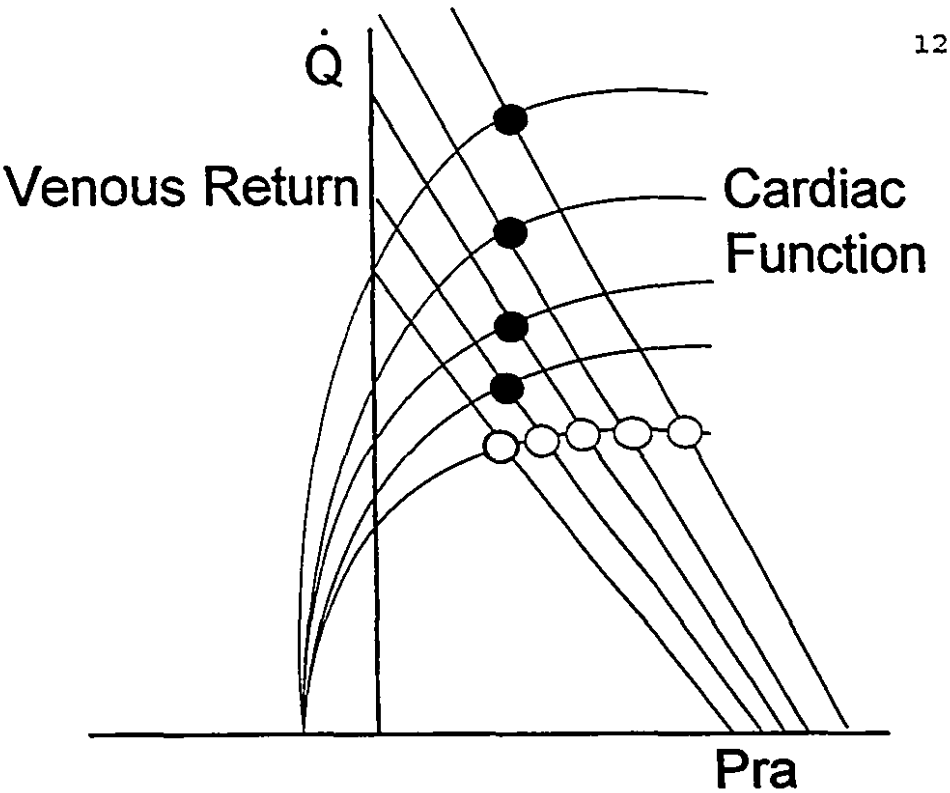

FIGURE 3.1. Schematic of cardiac response and venous return curves plotted on the same axis with both flows plotted vs right atrial pressure. Solid circles indicate the hypothesized steady state cardiac output during exercise resulting from changes in both cardiac and circuit response (upward shift in cardiac response curve, right atrial pressure does not change). The open circles indicate the hypothesized cardiac output during exercise resulting from changes in the venous circuit only (right shift in venous return curve and increased slope, right atrial pressure increases). See text for further details.

C. METHODS

Subject Preparation

Transplant Patients: Twelve male patients were recruited within the first year following orthotopic heart transplantation and gave informed consent to participate in the study. An additional two heart transplant patients were recruited for an inotropic drug protocol. The study was approved by the hospital's Ethics Committee. All patients were free from acute rejection and were receiving standard triple-dose immunosuppressive therapy consisting of cyclosporin, azathioprine and prednisone. Six of twelve patients were taking a calcium channel blocker and two others were on beta blockade medication, one of them on the test day only, to provide prophylaxis for arrhythmia during the biopsy. Mean time post transplant was 35.3± 12.5 (S.D.) weeks. None of the patients were taking part in a structured rehabilitation program. Patients were studied at the time of their routine rejection surveillance endomyocardial biopsy procedure. Following the biopsy, a Swan-Ganz catheter was inserted in the sheath already in place in the right external jugular vein, and advanced through the right heart into the pulmonary artery, using fluoroscopic guidance.

Normal Subjects: Six heathy males were recruited through advertisement at McGill University campus. All had negative medical histories, were not taking any medication and had normal resting electrocardiograms with the exception of one

who had evidence of Wolf-Parkinson White pattern but was asymptomatic. Informed consent was obtained and maximal exercise screening tests were performed on separate days. On the day of the test, an elongated antecubital venous catheter was placed at the junction of the superior vena cava and the right atrium. This position was verified with fluoroscopy. Healthy young subjects were selected as a comparison group in order to document the normal response of central venous pressure and cardiac output during identical levels of exercise on the same ergometer. Therefore we compared the responses of the heart transplant patients to the "ideal" or young, healthy normal response. Descriptive data on both subject groups are given in Table 3.1.

Exercise Protocol

The central catheter was placed with subjects in the supine position and they were then raised to an upright position of 80 degrees. Following resting measurements, subjects began cycling at a rate of 60 rpm and a power output of 25 watt. The power output was increased by 25 watt every 3 minutes until a maximal or peak effort. The latter was defined as the inability to continue pedalling at a rate of 60 rpm despite encouragement, combined with a respiratory exchange ratio of greater than 1.2.

Measurements

Right atrial (Pra), pulmonary artery (Ppa) and wedge (Pw) pressures in patients and central venous pressure (CVP) in

TABLE 3.1. Physical Characteristics of Heart Transplant (HT) Patients and Normal Subjects. Hb, hemoglobin; HR, heart rate; $\dot{V}O_2$, oxygen consumption. (Mean ± S.E.), *, $p<.05$.

	HT n=12	NORMALS n=6
Age (years)	51.2 ± 4.4*	22.7 ± 0.8
Height (cm)	169.3 ± 2.4	176.17 ± 1.87
Weight (kg)	73.1 ± 2.8	73.1 ± 3.22
Hb (gm/l)	139 ± 3.7	140.3 ± 4.7
Rest HR (bt/min)	97 ± 5.0*	67.2 ± 1.85
Peak HR	147 ± 6.9*	187.2 ± 2.5
Rest $\dot{V}O2$ (ml/min)	200.7 ± 12.4	264 ± 22.3
Peak $\dot{V}O2$	1257 ± 133*	4096.8 ± 284.9
Rest lactate(mmol/l)	0.85 ± 0.12	0.88 ± 0.2
Peak lactate	6.54 ± 0.63	8.48 ± 1.7

normal subjects were measured with transducers (Abbott Critical Care Systems, Abbott Lab.,N.Chicago,Il.) with output to a 2-channel recorder. Measurements were made at rest, during the third minute of each exercise stage and at 3 minutes of recovery. All pressures were measured at the end of expiration. The zero pressure reference point was set at 5 cm below the sternal angle, which is the junction of the second rib and sternum. The zero reference of the transducers was re-adjusted when the upright posture was assumed. The control subjects performed a brief breath-hold at end expiration during exercise while CVP was recorded in order to minimize the interference which occurred with very high respiratory rates. They were trained prior to exercise to avoid recruitment of abdominal muscles during this manoeuvre. This was not necessary with HT patients due to their reduced peak exercise capacity.

Heart rate was measured from the electrocardiogram at rest, after each minute of exercise, at peak effort and during recovery. We measured blood pressure at rest, during each exercise stage and at 3 minutes of recovery with a sphygmomanometer. Arterial saturation was obtained from an oximeter with finger probe.

We sampled venous blood from the central catheter at rest, at each exercise level and at 3 minutes of recovery for measurement of blood gases, oxygen saturation and lactate.

We obtained expired gases breath by breath to measure

oxygen consumption (VO_2) at rest and every 30 seconds during exercise and recovery by means of a computerized metabolic unit (Medgraphics™ CPX, Medical Graphics Corp., Minniapolis, MN). Subjects breathed through a low resistance one-way valve which was connected via a pneumotachygraph to the unit. Ventilatory parameters were recorded every 30 seconds.

Cardiac output was measured by thermodilution (the average of 3 measures within 10% of each other) and direct Fick calculation in patients. Cardiac output (CO) was calculated in control subjects from the following regression equation based on thermodilution measurements made during graded cycle exercise in healthy young males at sea level by Reeves et.al. (37):

$$CO=.00556\dot{V}O_2 + 5.87.$$

We were unable to perform direct Fick calculations in these subjects since the oxygen content of venous blood sampled outside the right atrium was not representative of mixed venous values.

Measurements were made in the following order during the last 1.5-2 minutes of each exercise stage: Blood pressure, central pressures, thermodilution cardiac outputs (in patients only), venous blood sample.

Dobutamine Protocol: Two additional heart transplant patients were studied during peak exercise in a similar manner with and without the infusion of 10μg/kg min of the synthetic catecholamine dobutamine (Eli Lilly & Co.,Indianapolis,IN) in

order to examine the effect of increasing cardiac function on exercise tolerance and peak oxygen consumption. These subjects began exercise at 50 watt and the power output was increased by 25 watt every 3 minutes until peak effort was achieved, as previously defined. The dobutamine infusion was then begun after 30 minutes of rest and was increased from 2.5 to 10μg/kg min over 10 minutes. The exercise protocol was then repeated.

Statistical Analysis

We compared physical data and resting and peak exercise responses of transplant patients and control subjects using Student t-tests for unpaired data. Linear regression analysis was used to evaluate the cardiac output/oxygen consumption relationship in HT patients.

Exercise data over time were analyzed for each group by means of a one-way analysis of variance for repeated measures. Main effects were considered statistically significant at the 95% confidence level and a post-hoc Newman-Keuls test was applied to assess differences between means. All data are reported as mean ± standard error.

D. RESULTS

Descriptive Data: Physical and descriptive data are presented in Table 3.1. The transplant patients were significantly older than the normal subjects. There was no significant difference in height, weight, hemoglobin concentration or resting oxygen uptake between groups. Resting

heart rate was higher in HT as expected from the denervated state ($p<.05$). Peak heart rate, work rate and $\dot{V}O2$ were higher in the normal group. ($p<.05$). Peak $\dot{V}O2$ of the transplants averaged 57% of the predicted value for their age (1). There was no difference in resting or peak venous blood lactate between the two groups.

Cardiac Output/$\dot{V}O_2$ Relationship: Figure 3.2 shows the individual resting and exercise values of cardiac output vs oxygen consumption ($\dot{V}O_2$) of the 12 HT patients. The regression equation that describes this relationship is: $CO = .00597\ \dot{V}O_2 + 4.4$ ($r=0.83$).

Hemodynamics: Both groups had a significant and similar increase in cardiac output (CO) with each increment in power output ($p<.0001$). However, the transplant patients had significantly higher right atrial pressures (Pra) during exercise compared with normals (Figure 3.3A). Both groups showed a significant increase in Pra in the transition from rest to exercise ($p<.05$) but the normal subjects had little further increase in Pra with increasing cardiac output. In contrast, Pra increased significantly during exercise in the transplant group (8.9± 1.6 and 12.6± 1.8 mmHg at 25 and 50 watt, $p<.05$) but stabilized at higher work rates (13.5± 1.6 mmHg at 75 watt, N=4) as cardiac output continued to increase (Figure 3.3A). There was an initial large increase in pulmonary artery and wedge pressures in the transition from rest to 25 watt but little further increase as exercise

continued (Figure 3.4). Resting, submaximal and peak data are given in Table 3.2.

The relationship of stroke volume to Pra is shown in Figure 3.3B. In normal subjects, during exercise up to 100 watt, there was no significant increase in stroke volume with the small change in Pra. Stroke volume was significantly increased from baseline ($p<.05$) only during the last 3 power outputs. In contrast, stroke volume in the transplant group was significantly increased at each exercise level ($p<.05$). Patients had a lower resting stroke volume compared with the normal subjects ($p<.0001$), consistent with a higher resting heart rate due to denervation. However, by 75 watts the 2 groups had similar stroke volumes (HT=120.5± 20.1 and control=132.5± 9.3 ml).

Dobutamine: Exercise time and peak VO_2 remained almost identical with or without infusion of dobutamine in 2 patients in spite of a normalization of the peak heart rate response. There was a large increase in peak cardiac output with dobutamine in one patient (12.7 to 18.6 l/min) and a smaller increase in the other patient (15.2 without to 17.2 l/min with dobutamine). Pra was reduced by approximately 6 mmHg at peak exercise with dobutamine vs control in both patients. Individual data are given in Table 3.3.

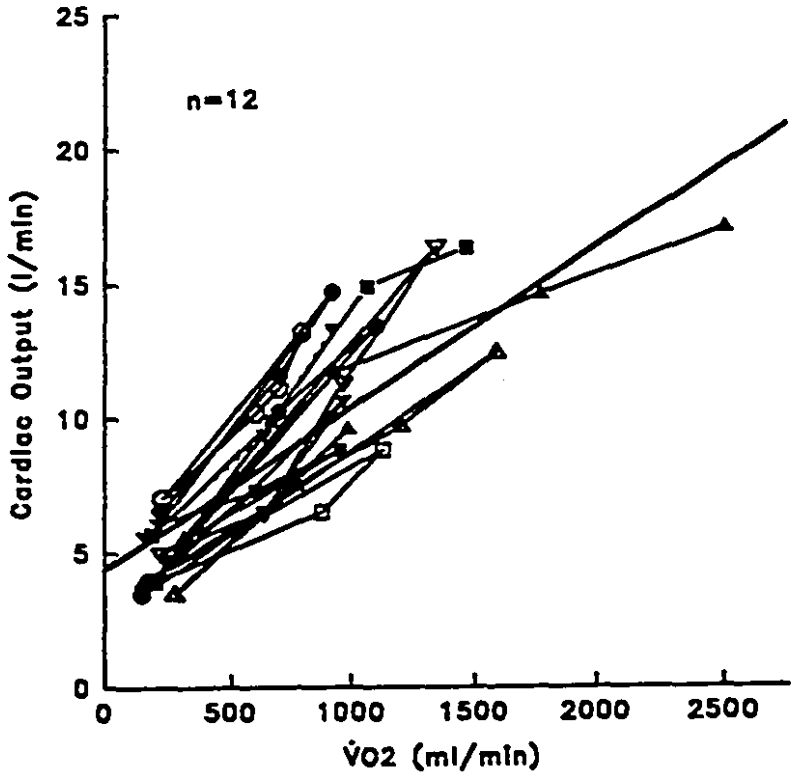

FIGURE 3.2. Individual cardiac output values (CO) (l/min) plotted against oxygen consumption ($\dot{V}O_2$) (ml/min) at rest and during exercise in individual heart transplant (HT) patients (N=12). Thick solid line between y-axes is the linear regression line with the equation: $CO = 0.00597\dot{V}O_2 + 4.4$, r= 0.83.

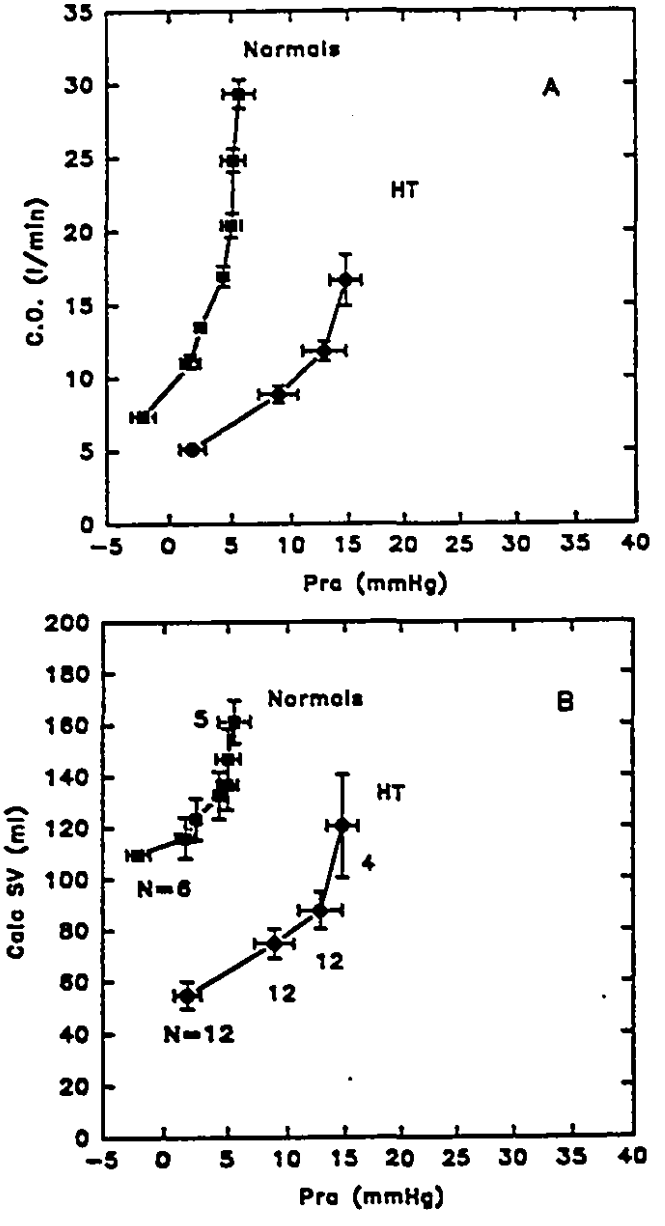

FIGURE 3.3. A. Cardiac output (CO) (l/min) vs right atrial pressure (Pra) (Mean± S.E.) in normal subjects (solid squares) and heart transplant patients (HT) (solid circles). B. Calculated stroke volume (SV) (ml) vs right atrial pressure (Pra) (mmHg) (Mean± S.E.) at rest and during upright exercise in normal subjects and HT patients.

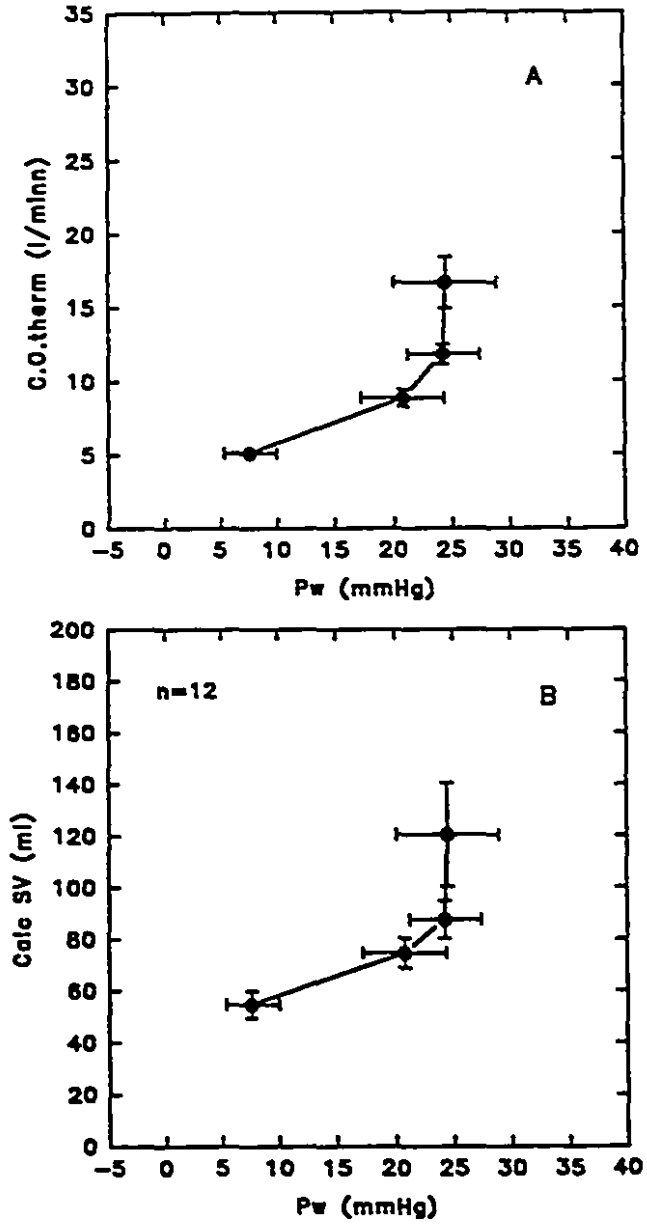

FIGURE 3.4. A. Cardiac output (CO) (l/min) vs pulmonary wedge pressure (Pw) (mmHg) (Mean± S.E.) at rest and during upright exercise in heart transplant patients (HT) (solid circles). B. Calculated stroke volume (SV) (ml) vs pulmonary wedge pressure (Mean± S.E.) in HT patients.

TABLE 3.2 Rest and Exercise Hemodynamic Data for Heart Transplant (HT) Patients and Normal (Norm) Subjects. Ppa, pulmonary artery pressure; Pw, pulmonary wedge pressure; a-$\bar{v}O_2$, arterio-mixed venous oxygen difference; CO, cardiac output; $\dot{V}O_2$, oxygen consumption; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure. *, $p<.05$ from rest; +, $p<.05$ from other exercise values.

	Sit rest	25 W	50 W	75 W	100 W	125 W	150 W	peak
HT								
Ppa mmHg	16.5± 2.9	35.0± 3.8*	40.2± 4.3*	40.8± 8.5*	----	----	----	41.9±4 .0
Pw mmHg	7.7± 2.3	20.8± 3.6*	24.3± 3.1*	24.5± 4.4*	----	----	----	24.5±3 .1
a-$\bar{v}O_2$ ml/l	51.4± 2.1	71.6± 3.3*+	90.7± 5.9*+	107.6± 11.6*	----	----	----	97.1±6 .9
CO l/min								
HT	5.1± 0.3	8.9± 0.6*+	11.8± 0.7*+	15.5± 1.1*+	----	----	----	13.0± 0.9
Norm	7.3± 0.1	11.0± 0.6*+	13.5± 0.4*+	16.9± 0.7*+	20.4± 0.8*+	24.8± 0.8*+	29.3± 1.0*+	28.0± 1.5
$\dot{V}O_2$ml/m in								
HT	211.3± 13.7	724.5± 27.2*+	1074± 70.8*+	1731± 264*+	----	----	----	1257± 133
Norm	264± 22.3	923.7± 100*+	1369± 75.1*+	1991± 120.7*+	2617± 148.3*+	3405± 139.0*+	4216± 177*+	4097± 284.9
HR bt /min								
HT	98± 5.0	122.8± 6.4*+	140.8± 6.9*+	139± 13.2*	----	----	----	147± 6.9
Norm	67.2± 1.9	96± 4.2*+	111.2± 6.1*+	130± 7.0*+	152± 8.1*+	172.7± 9.4*+	183± 6.1*+	187.2± 2.5
SBP mmHg								
HT	146± 6.3	154.5± 10.0*+	173.5± 10.3*	187.0± 20.3*	----	----	----	181.3± 11.0
Norm	115.3± 5.7	122.3± 5.4+	135.8± 3.7*+	156.7± 5.4*	163.3± 4.0*	181.7± 5.3*	192± 4.9*	190.8± 4.5
DBP mmHg								
HT	90.3± 3.9	89.0± 4.5	87.8± 4.7	72.5± 18.4	----	----	----	88.4± 5.4
Norm	73.3± 3.3	75.0± 3.4	70.8± 3.3	75.0± 2.2	75.0± 3.4	71.7± 4.2	78.0± 2.0	74.2± 4.2

TABLE 3.3. Dobutamine Protocol. Comparison of Individual Values at Peak Exercise in Two Patients with Saline vs Dobutamine Infusion.Table 3 Dobutamine Protocol

Patient 1	HR bt/min	$\dot{V}O_2$ ml/min	exercise time (min)	CO l/min	a-vO_2calc ml/l	CVP mmHg	Ppa	Pw	%ven sat	SBP mmHg	DBP	RER	La mmol\l
saline	130	1224	4.5	12.7	96.4	8	26.6	11	N\A	174	98	1.4	N/A
dobutamine	158	1308	4.5	18.6	70.3	1.5	19	N/A	N/A	174	80	1.3	N/A
Patient 2	**HR bt/min**	**$\dot{V}O_2$ ml/min**	**exercise time (min)**	**CO l/min**	**a-vO_2calc ml/l**	**CVP mmHg**	**Ppa**	**Pw**	**%ven sat**	**SBP mmHg**	**DBP**	**RER**	**La mmol\l**
saline	140	1709	11.5	15.2	112.4	17.5	32	16	47	174	80	1.4	11.1
dobutamine	168	1826	11.5	17.2	106.2	12	30	10	48	170	90	1.3	9.3

HR, heart rate; $\dot{V}O_2$, oxygen consumption; CO, cardiac output; a-vO_2calc, calculated arterio-venous oxygen difference; CVP, central venous pressure; Ppa, pulmonary artery pressure; Pw, pulmonary wedge pressure; % ven sat, % venous oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; RER, respiratory exchange ratio; La, venous lactate; N/A, not available.

E. DISCUSSION

The main findings of this study are: 1) Heart transplant recipients have normal cardiac output responses to increasing oxygen demands during steady state exercise; 2) Based on the observed increase in cardiac output and stroke volume with little change in right atrial pressure at peak exercise, these patients do not appear to be limited by their denervated heart. This suggests both chronotropic and inotropic reserves at peak exercise.

The heart transplant patients in this study showed the typical response to graded exercise as reported by others: a blunted increase in heart rate, large increase in central pressures, particularly in the rest to exercise transition and a decrease in peak heart rate and oxygen consumption (2,23-25,32,34,39,40,42,48).

Cardiac Output Response: The equation describing the cardiac output/oxygen consumption relationship of the transplant patients, shown in Figure 3.2, is very similar to previous reports of normal individuals (13,14,23,25,37). The intercept of 4.4 l/min is slightly lower than that observed in studies on young normals, but is similar to the slightly lower intercept observed by both Granath et al.(15) and McElvaney et al. (29) in normal, older individuals. Therefore, these heart transplant patients had a normal increase of cardiac output with increasing oxygen consumption which is similar to other reports (7,8,23). Stinson and others (6,43) interpreted

similar findings during supine exercise to indicate a central cardiac limit to exercise resulting from impaired chronotropic response due to denervation, but they did not consider the age effect in this relationship. This view dominated the early literature in this area in spite of canine studies which showed that denervation alone resulted in little functional impairment during maximal exercise (10,11).

Others have also concluded that the blunted heart rate response represents the major limitation to exercise in HT patients (40,42). Kao et al. recently noted that the decreased peak cardiac output achieved by HT vs age-matched controls indicates a cardiac limitation (24). However, they showed that cardiac output response was normal at a given level of oxygen consumption. Since cardiac output and oxygen consumption are normally very tightly matched (12-14,23,25), with the cardiac output as the dependent variable, it is most likely that the decreased peak cardiac output is due to decreased peak oxygen consumption because of peripheral limitation rather than the reverse.

In spite of blunted heart rate responses to exercise, our patients were able to maintain a normal cardiac output for a given metabolic demand during graded exercise, but achieved only 57% of their age-predicted peak oxygen consumption. We assessed the relative contribution of cardiac and peripheral vascular factors and how these factors interact by examining the steady state cardiac output/right atrial pressure

relationship during graded exercise. These points (Figure 3.3A) are the intersection points of the cardiac function and venous return curves (see Figure 3.1).

Cardiac Output/Right Atrial Pressure Relationship: Control subjects showed small increases in SV with small increases in Pra during exercise, which demonstrates their reliance on increases in heart rate and contractility to increase cardiac output. In contrast, the HT patients showed significant increases in SV at each work level, with significant increases in filling pressure, particularly in the transition from rest to exercise (Figure 3.3B). This is consistent with a reliance on the Frank-Starling mechanism to increase cardiac output as compensation for the blunted heart rate response, as has been shown by others during both supine and upright exercise (34,48). The steep increase in cardiac output vs Pra as the heart transplant patients approached peak exercise was similar to that seen in the normal subjects, albeit at a higher filling pressure or Pra equilibrium point (Figure 3.3A). This is similar to the closed circles in Figure 3.1 and indicates that cardiac contractility must have increased with increasing work effort even in the HT patients. This was most likely due to increases in circulating catecholamines which accumulate as exercise continues and allow cardiac function to keep up with increases in venous return (2,34,48). The cardiac response/venous return intersection points are shifted to the right in the patients compared with normals due to the delayed

adaptations to graded exercise in HT patients. In normal subjects, these changes occur simultaneously, because of the tight coupling of the sympathetic response to the effort and there is little change in Pra.

The increase in central pressures of HT patients during exercise that we observed is similar to a previous report in which a similar exercise protocol was used (40) although the mean peak pulmonary wedge pressure was lower in that study (18±8 vs 24.5±4.4 mmHg in our study). This may reflect the increased age of our patients. Despite the high pulmonary pressures, patients did not show significant arterial oxygen desaturation. The high pulmonary pressures have been attributed to a mismatch between cardiac response (i.e. heart rate and contractility) and venous return as well as to a reduced ventricular compliance due to impaired ventricular relaxation or a relatively small donor heart size (22,32,39).

Whereas reduced ventricular compliance of HT patients during exercise has recently been demonstrated (24), the interaction of a delayed increase in cardiac response and a normal increase in venous return can result in an increase in filling pressure during exercise which would put the heart on the noncompliant part of its pressure/volume relationship and make it functionally noncompliant. However, this did not seem to limit exercise because there was no plateau in cardiac output at peak exercise in Figure 3.3A. This argues against a cardiac limit. In fact, the steep increase in cardiac output

and stroke volume relative to Pra during severe exercise suggests that cardiac function increased until the end of exercise.

Dobutamine: As we could not rule out that our patients may have reached their contractile limit at peak exercise, we administered the inotropic drug dobutamine to two additional patients. We observed an increase in cardiac output and a decrease in right atrial pressure of about 6 mmHg but no increase in exercise time. Peak oxygen consumption was slightly increased in both patients, most likely due to a catecholamine-induced increase in metabolism. This suggests that heart transplant patients have cardiac contractile reserves at peak exercise and the heart is not limiting their exercise performance.

Recruitment of cardiac reserve at peak exercise with dobutamine, however, did not increase exercise time. A similar observation has been noted in patients with severe chronic heart failure (28,47) as well as in conscious exercising dogs (20). However, they reported similar peak oxygen consumption with dobutamine associated with increased cardiac output as well as increased mixed venous oxygen content, compared to control. This is consistent with an altered distribution of excess blood flow away from exercising muscles. We found little change in mixed venous oxygen saturation at peak exercise in one patient during dobutamine infusion. Furthermore, our data show that the heart still

has the capacity to increase its output at peak exercise in these patients. Thus, the lack of increase in exercise capacity with increased cardiac function argues against the heart as the major limiting factor to exercise in heart transplant patients.

The question then remains as to what limits the exercise capacity of heart transplant patients. There are a number of possibilities: 1) a reduction in skeletal muscle mass and function which could be secondary to deconditioning (5,29); 2) pulmonary congestion from the high pulmonary wedge pressures during exercise, although our patients continued to exercise with high pulmonary pressures and did not desaturate; 3) muscle congestion from the high central venous pressures during exercise which suggest even higher venous pressures in muscle, although the dobutamine data would argue against this; 4) a build up of lactate in muscle, not reflected in measured blood levels, which may result from a delayed increase in muscle perfusion. Muscle lactate levels have not been directly measured in this patient population.

Study Limitations: We were unable to directly measure cardiac output in the control subjects since the central catheter was not advanced far enough to reliably sample mixed venous blood. However, by applying an equation derived from a similar group of individuals, in whom cardiac output was measured by thermodilution as in our patients, we could predict cardiac output during exercise stress. This is

supported by the tight relationship between cardiac output and oxygen consumption which allows prediction of the former from the latter in normal subjects (14).

In the dobutamine protocol, we assumed that the dose used would result in a significant increase in cardiac contractility during exercise. However, as catecholamines were not measured, the dose may have been too small, relative to circulating catecholamines, to have made a detectable difference in performance. We did observe a normalization of peak heart rate with dobutamine which has been shown to improve cardiac contractility in both coronary artery bypass graft patients and heart transplant recipients (38).

Conclusions: We conclude that the reduced exercise tolerance of heart transplant patients is not simply due to cardiac limitations. Peripheral factors related to skeletal muscle function and blood flow may be more important limits to exercise in patients following heart transplantation.

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CHAPTER 4

Central Venous Pressure During Exercise:

Role of Muscle Pump

Chapter Link

The previous study used the cardiac output/right atrial pressure relationship to assess the interaction between heart and periphery and which limited peak oxygen consumption in heart transplant patients. Right atrial pressure or central venous pressure is the common variable linking cardiac and peripheral circuit function and reflects alterations in both during exercise. Although there was little change in steady state central venous pressure in normal subjects during exercise in the preceding study, I noted an immediate increase at the commencement of exercise. Previous studies have attributed this to a shift of peripheral to central blood volume as a result of the pumping action of the muscles. Therefore, in the next study I examined central venous pressure immediately at exercise onset and after a few minutes of exercise to assess the mechanical effect of muscle contraction compared with neurally-mediated changes in cardiac function in heart transplant and normal subjects. In this context, heart transplant patients provide a model for isolating peripheral changes and their influence on central venous pressure from changes in cardiac function at the onset of exercise.

A. ABSTRACT

The sudden increase in central venous pressure (CVP) observed at the onset of dynamic exercise has been attributed to the action of the muscle pump but is also affected by reflex changes in cardiac response. CVP gives the integral result of changes in cardiac and peripheral factors. To determine which is predominant at the start of exercise, we compared the change in CVP from rest to onset of upright exercise and after 3 minutes of exercise in 4 healthy normal subjects (N) to 6 patients following heart transplantation (HT), who thus had a delayed cardiac response. CVP immediately increased to a similar extent in both groups at exercise onset (by 4.6±0.6 in HT and 4.0±0.4 mmHg in N) (Mean±S.E.). After 3 minutes of exercise, CVP remained constant in N (2.0±1.1 vs 2.6±1.8 at onset), but increased further in HT (7.0±0.8 vs 3.5±1.1 at onset, $p<.05$). The immediate increase in CVP with leg movement in both supports a central shift in blood volume due to muscle contraction. As exercise continues, CVP increases further in HT but not in N due to the slower component of reflex cardiac adjustment in HT. We conclude that the muscle pump increases CVP at exercise onset but the interaction between cardiac response and circuit function determines CVP as exercise continues.

B. INTRODUCTION

The normal heart can adjust to the increase in venous return during exercise in two ways. One is through the Frank-Starling mechanism by which the increase in the cardiac end-diastolic volume leads to an increase in stroke volume and cardiac output. This can be expressed graphically by movement upward along a cardiac function curve (6) (Figure 4.1). For cardiac filling to increase, there must be an increase in the return of blood to the heart (i.e. venous return) which implies a change in peripheral circuit factors. Graphically, this is represented by either a shift in the venous return curve or change in its slope (6) (Figure 4.1).

The other way is through an increase in cardiac function which can be due to an increase in contractility or an increase in heart rate. This can be graphically represented by an upward shift of the whole cardiac function curve. Obviously, both can occur simultaneously, but when the increase in cardiac output is predominantly due to increased cardiac filling pressures and the Frank-Starling mechanism, the CVP will increase (point B in Figure 4.1). On the other hand, when the increase is predominantly due to an increase in pump function, the CVP will fall (point A in Figure 4.1). If cardiac output increases and CVP remains constant, the adaptation must have occurred in both the heart and circuit and these changes must be of similar magnitude. The simple measurement of CVP, in a condition where cardiac output is

known to increase, can give important information about the interaction of the heart and circuit.

Cardiac output increases at the start of exercise. In normal subjects this could be due to neurally mediated rapid adjustments in cardiac function as well as changes in the circuit function with increased cardiac filling. A factor which has recently received a lot of attention and could cause an immediate increase in venous return before any neuro-humoral adjustments, is the action of muscle contractions which produces an effective pump for the return of blood to the heart. This acts before neuro-humoral factors, thus CVP should rise immediately. Patients who have undergone transplants have denervated hearts. They then cannot have an immediate neural adjustment, although they are known to have a slower but effective humoral adjustment during exercise. However, the muscle pump mechanism should be the same and CVP should also rise at the onset of exercise.

The simple measurement of CVP at the onset of exercise thus gives important insight into the interaction of the heart and peripheral circuit. By comparing normal subjects to patients with heart transplants, we were able to examine the CVP change at the onset of exercise in subjects with and without cardiac innervation. This allowed a comparison of normal subjects to a group in whom there could not be an immediate neural response. If the muscle pump mechanism predominates at the onset of exercise, there should be an immediate and similar

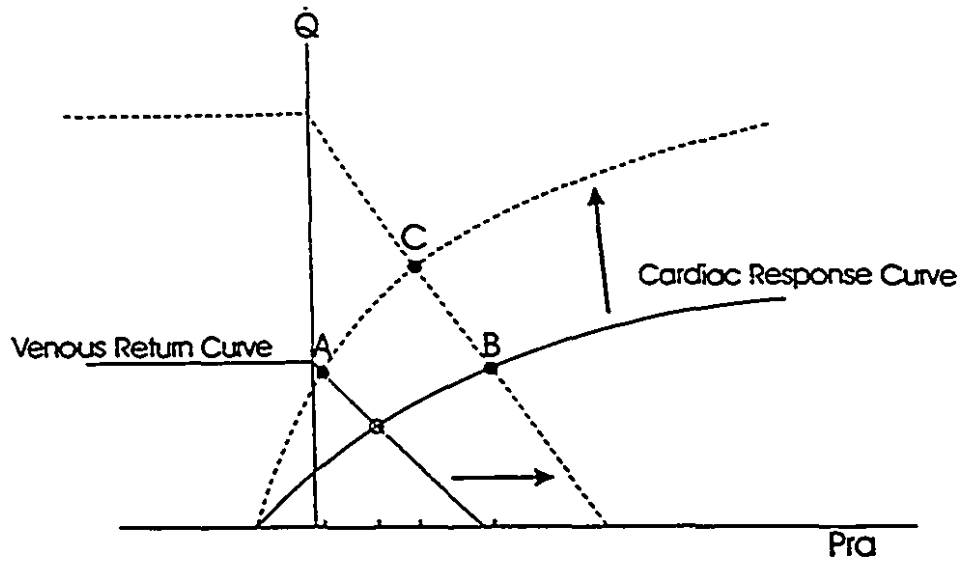

Figure 4.1. Schematic of cardiac response and venous return curves plotted on the same axis with both flows plotted vs right atrial pressure. Solid lines indicate normal resting curves. Broken lines denote shifted curves. Hollow circle indicates the normal resting steady state cardiac output or venous return. Solid circles indicate: the hypothesized steady state cardiac output (Point A) during exercise resulting from changes in cardiac response only (left shift in cardiac response curve, right atrial pressure decreases); the hypothesized cardiac output during exercise resulting from changes in the venous circuit only (Point B) (right shift in venous return curve and increased slope, right atrial pressure increases); and the cardiac output resulting from shifts in both cardiac response and venous return curves during exercise (Point C) (right atrial pressure is the same as rest or slightly increased). See text for further details.

rise in CVP in normals and HT patients. However, after a few seconds, this component should be "masked" in normal but not HT patients by the neurally mediated increase in cardiac responses.

C. METHODS

Subject Preparation

Control Subjects: We recruited four healthy males through advertisement at McGill University campus and obtained informed consent. All had negative medical histories and were not taking any medication. We obtained informed consent and the subjects performed maximal exercise screening tests on both the treadmill and cycle ergometer on separate days. On the day of the test, an elongated antecubital venous catheter was placed at the junction of the superior vena cava and the right atrium. This position was verified with fluoroscopy.

Transplant Patients: Six male patients were recruited within the first year following orthotopic heart transplantation and gave informed consent to participate in the study. The study was approved by the hospital's Ethics Committee. All patients were free from acute rejection and were receiving standard triple-dose immunosuppressive therapy consisting of cyclosporin, azathioprine and prednisone. Four patients were taking calcium channel blockers and one was on beta blockade medication on the test day only as a prophylaxis for arrhythmia during the biopsy. Mean time post transplant was

29.6 ± 10.6 (S.D.) weeks. Patients were studied at the time of their routine rejection surveillance endomyocardial biopsy procedure. Following the biopsy, a Swan-Ganz catheter was inserted in the sheath already in place in the right external jugular vein, and advanced through the right heart into the pulmonary artery, using fluoroscopic guidance. Descriptive data on both subject groups is given in Table 4.1.

Testing Procedures

Resting measures of CVP, heart rate and ventilatory parameters were made in the upright position. The zero pressure reference point was 5 cm below the sternal angle, which is the junction of the second rib and sternum. The zero reference of the transducers was re-adjusted when the upright posture was assumed. A power output of 25 watts was applied approximately 4 seconds after the subject began pedalling at a rate of 60 rpm.

Therefore, the first few revolutions were at zero load. Subjects continued to exercise at 25 watts for 3 minutes. Right atrial pressure and pulmonary wedge pressure in patients and CVP in control subjects were measured at the end of expiration at the onset of pedalling and after 3 minutes of exercise. Heart rate was measured from the electrocardiogram at rest, during the first 5 seconds and after 3 minutes of exercise. We obtained expired gases breath by breath to measure oxygen consumption at upright rest, at 30 seconds following exercise onset and at 3 minutes of exercise by means

TABLE 4.1. Descriptive data. Mean ± Standard Error of age, height, weight, hemoglobin concentration (Hb) and peak oxygen consumption ($\dot{V}O_2$) in normal subjects (n=4) and heart transplant patients (n=6). *, significant difference from normal subjects ($p<.05$).

	Normal n=4	**Heart Transplant** n=6
Age (years)	22.5 ± 0.3	46.7 ± 7.9*
Height (cm)	175.3 ± 2.8	169.0 ± 4.3
Weight (kg)	68.3 ± 0.9	67.8 ± 3.9
Hb (gm/L)	135.8 ± .03	137.8 ± 0.6
Peak $\dot{V}O_2$ (ml/min)	3834.3 ± 309.9	1368.5 ± 250.2*

of a computerized metabolic unit (Medgraphics™ CPX, Medical Graphics Corp., Minniapolis, MN). Subjects breathed through a low resistance one-way valve which was connected via a pneumotachometer to the unit.

Cardiac output was measured at rest and during the third minute of exercise by thermodilution (the average of 3 measures within 10% of each other) in patients. Cardiac output (CO) was calculated in control subjects from the following regression equation based on thermodilution measurements made during graded cycle exercise in healthy young males at sea level by Reeves et.al.(1987):

$$CO = .00556\dot{V}O_2 + 5.87.$$

We were unable to perform direct Fick calculations in these subjects since the oxygen content of venous blood sampled outside the right atrium was not representative of mixed venous values.

Statistical Analysis

Data was analyzed for each group by means of a one-way analysis of variance for repeated measures. Main effects were considered statistically significant at the 95% confidence level and a post-hoc Newman-Keuls test was applied to assess differences between means. We compared the increase from baseline at onset and at 3 minutes of exercise across groups by paired t-test. All data are reported as mean ± standard error.

D. RESULTS

An example of the experimental record in a normal and HT subject is shown in Figure 4.2. CVP increased immediately upon commencement of pedalling in both control and transplant subjects and to a similar degree. Mean values ± standard error for CVP, heart rate, minute ventilation and oxygen consumption at rest, at exercise onset and at 3 minutes of exercise in both groups are given in Table 4.2.

Central Venous Pressure: There was a significant main effect of time on CVP ($F=12.13, df=2,6, p<.008$) in normal subjects. CVP at exercise onset and at 3 minutes of exercise were not different but both were significantly greater than rest ($p<.05$) (Figure 4.3).

Heart Transplant patients also had a significant main effect of time on CVP ($F=93.89, df=2,10, p<.0001$). However, mean CVP at 3 minutes of exercise was higher than at exercise onset and both were greater than rest ($p<.05$) (Figure 4.3). The increase in CVP from rest to 3 minutes was greater than the increase at exercise onset ($t=-4.87, df=5, p<.005$). Pulmonary wedge pressure during the third minute of exercise was also significantly elevated from rest ($p<.001$) but was not available for the immediate onset of exercise. Mean values are shown in Table 4.2.

Heart Rate: Heart rate increased from rest to the onset of exercise in normals ($p<.05$) but not in transplant patients (Table 4.2). However, heart rate increased in the transplant

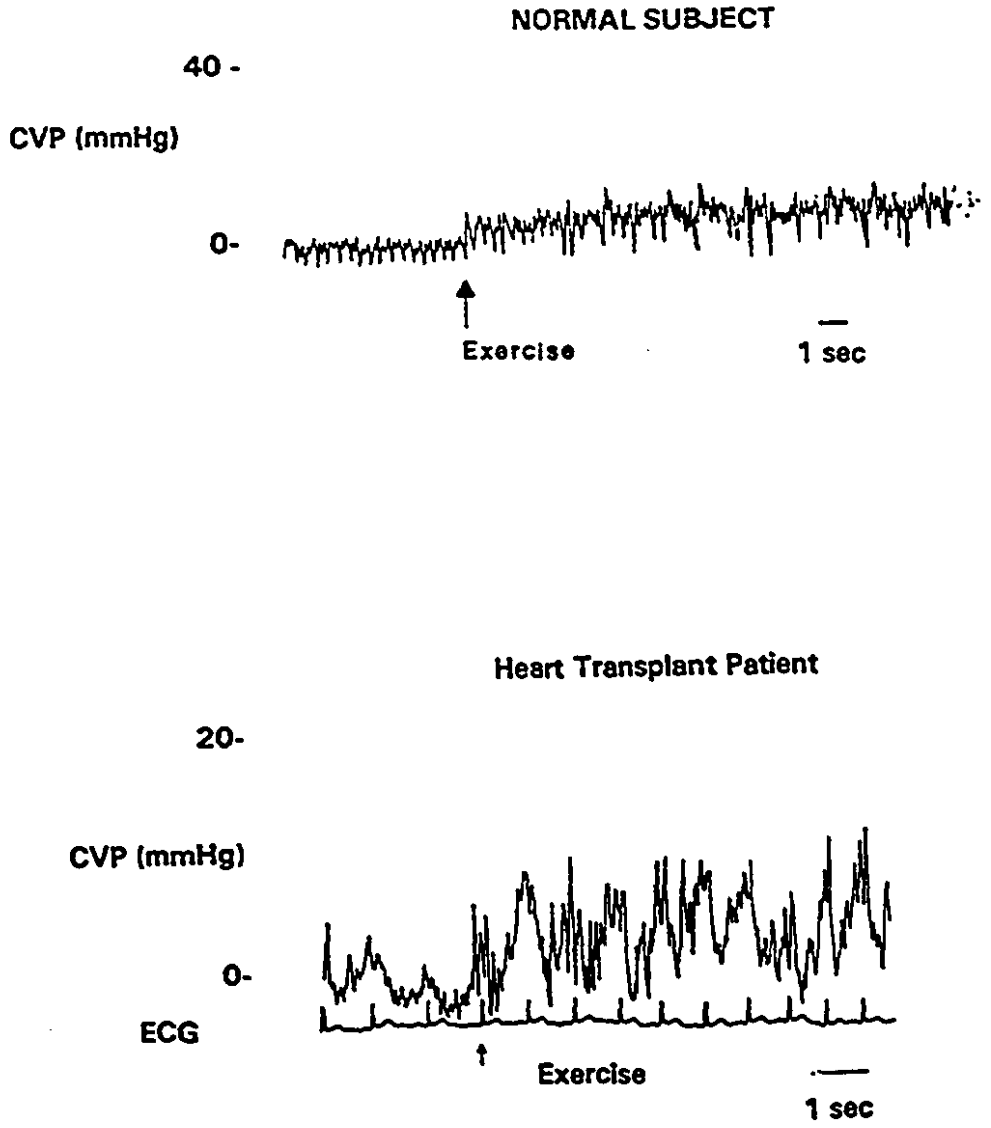

Figure 4.2A. Example of central venous pressure (CVP) trace from normal subject during transition from rest to cycle exercise at 25 watts. B. Example of right atrial pressure trace from heart transplant patient during transition from rest to cycling exercise at 25 watts.

TABLE 4.2. Mean ± Standard Error of central venous pressure (CVP), heart rate (HR), oxygen consumption ($\dot{V}O_2$) and minute ventilation ($\dot{V}_E$) in normal subjects (Norm, n=4) and heart transplant patients (HT, n=6). *, significant difference from rest ($p<.05$); +, significant difference from exercise onset ($p<.05$).

		Sitting Rest	Exercise Onset	3 min.Exercise
CVP(mmHg)	Norm	-2.0 ± 1.5	2.6 ± 1.8*	2.0 ± 1.2
	HT	-0.5 ± 0.9	3.5 ± 1.1*	7.0 ± 0.8*+
HR (bpm)	Norm	67.0 ± 2.7	75.0 ± 2.3*	97.3 ± 1.5*+
	HT	104.2 ± 5.8	109.2 ± 6.5	130.5 ± 9.2*+
$\dot{V}O_2$	Norm	261.0 ± 35.1	444.8 ± 50.7	970.8 ±151.2*+
(ml/min)	HT	213.2 ± 28.0	368.0 ± 57.8*	730.8 ± 45.1*+
CO(l/min)	Norm	7.32 ± 0.2	----------	11.27 ± 0.84*
	HT	4.5 ± 0.42	----------	8.84 ± .78*
PWP (mmHg)	Norm	----------	----------	----------
	HT	7.83 ± 4.1	----------	23.6 ± 6.2*
$\dot{V}_E$(L/min)	Norm	10.5 ± 0.5	15.8 ± 1.1*	23.8 ± 1.9*+
	HT	12.7 ± 1.2	17.9 ± 1.4	34.2 ± 3.3*+

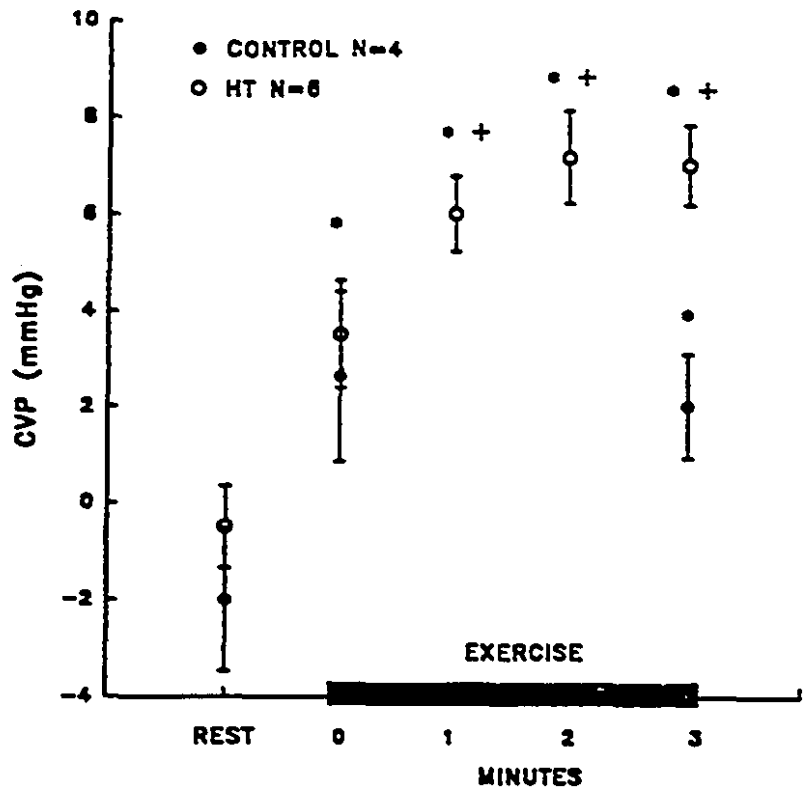

Figure 4.3. Central venous pressure (CVP) levels (Mean± S.E.) in mmHg at rest, exercise onset and during upright exercise at 25 watts on a cycle ergometer in normal subjects (solid circles) and heart transplant patients (open circles). *, values significantly different from control at p<.05; +, values significantly different from exercise onset at p<.05.

patients by 3 minutes of exercise ($p<.05$).

Cardiac Output: Heart transplant patients demonstrated an increase in cardiac output from rest to the third minute of exercise ($p<.003$). The predicted cardiac output of normal subjects, calculated from the known relationship of cardiac output and VO_2, demonstrated an appropriate rise ($p<.001$) and the values were similar to those measured in HT (Table 4.2). The resting VO_2 in normals was higher than in HT possibly due to a stronger anticipatory response.

E. DISCUSSION

Central venous pressure increased immediately in the transition from rest to cycling exercise in both heart transplant patients and healthy normal subjects and the mean increase was of the same magnitude in both groups, approximately 4 mmHg. However, as exercise continued, central venous pressure increased further in the transplant patients whereas it plateaued or tended to decrease in the normal subjects.

Before addressing the implications of this result, we must consider some technical factors related to the measurement of central venous pressure.

By measuring CVP relative to atmosphere and not intrathoracic pressure, the transmural pressure and therefore the effective filling pressure is unknown. Esophageal pressure has been measured in small numbers of subjects during upright

exercise to determine changes in pleural pressure (8,13). Holmgren (8) found no change in mean esophageal pressure during cycle exercise in 14 normal subjects but noted a small transitory increase at exercise onset in half of these subjects. Sprangers et.al. (13) showed no change in 2 subjects at the onset of cycle exercise and concluded that their reported change in right atrial pressure was thus equal to the change in right atrial transmural pressure. It is therefore unlikely that an increase in intrathoracic pressure explains the rise in CVP. The increase in CVP was sustained in our study.

We measured central venous or right atrial pressure at the end of expiration in order to control for respiratory variations. Most authors have used the electronic mean value of central venous pressure which is dependant on the magnitude of inspiratory and expiratory swings in pressure. Finally, care was taken to insure that the position of the subjects relative to the transducer was maintained to avoid movement artifact in the pressure measurement.

The lack of any discernable delay between the movement of the legs at exercise onset and the rise in CVP in both normal and transplant patients, as well as the small rise in heart rate of only 8 beats per minute in the normal group indicate that the response is unlikely to be due to neurohumoral mechanisms and is most likely due to a mechanical effect of muscle contraction on veins. Sheriff also found a mean

increase of approximately 5 mmHg when dogs began running on a treadmill with or without autonomic blockade (12). Muscle contractions may compress veins in exercising muscle as well as the abdomen. This effectively increases mean circulatory filling pressure which would increase the gradient for venous return. The magnitude of this increase in average mean circulatory filling pressure during muscle stimulation in anesthetized dogs with sympathetic ganglionic blockade with hexamethonium was from 3.8 to 11.3 mmHg and did not occur when contractions were blocked by a paralytic agent (7). This would result in a large volume shift toward the heart from peripheral capacitance vessels at exercise onset and result in an increase in central venous pressure. If we assume a venous compliance of 2-4 ml/mmHg·kg (4,7) and no change in venous resistance such that the change in CVP in our data represents the change in mean circulatory filling pressure, we can estimate the magnitude of volume mobilized by the muscle pump mechanism as 8-16 ml/kg at exercise onset. This corresponds to a significant immediate volume shift of 560-1120 ml in a 70 kg person. These numbers are consistent with the estimate of 9.5 ml/kg made in dogs at the onset of exercise (12). They are also consistent with the relative increase in central blood volume with a decrease in lower extremity blood volume, measured by radionuclide techniques, reported by Flamm and colleagues at the transition from rest to zero-load cycling in normal exercising humans (5). Others have reported a

significant decrease in venous pressure in the lower limbs as a result of a single muscle contraction, both in normal subjects and spastic paraplegics and concluded that regional venous volume is reduced and central blood volume increased (3,9).

Therefore the muscle contractions produce an immediate increase in the gradient for venous return. This mechanical effect is faster than reflex adjustments and provides an immediate increase in venous return at exercise onset which precedes neural adaptations. Thus the initial increase in cardiac output is most likely due to a predominant Frank-Starling mechanism.

Since changes in CVP or right atrial pressure are dependant on changes in cardiac response as well as changes in peripheral circulatory factors (7), it is not surprising that after 3 minutes of exercise, CVP in normal subjects was the same or slightly lower than at exercise onset. By this time, normal reflex changes in both the heart and periphery have occurred and the improvement in cardiac response has matched or slightly exceeded peripheral adaptations. Holmgren also described a trend for CVP to increase at work onset and then decrease as work continued (8). Both he and more recently, Reeves et.al. found little further increase in CVP with increasing work rates (11). Therefore, the normal reflex adaptation in cardiac response maintains CVP at a similar operating level after the initial increase at exercise onset.

However, in heart transplant patients, increases in cardiac response are delayed due to denervation and only occur through the slow increase in circulating catecholamines (1,2,10). Thus, CVP continued to increase due to the increasing venous return in combination with a blunted reflex change in cardiac response. The further rise in CVP, following the immediate onset of exercise in these patients, therefore provides a measure of the effects of peripheral reflex changes combined with blunted cardiac changes on CVP during dynamic exercise.

Our data thus suggest that the action of the muscle pump accounts for the increase in CVP immediately at exercise onset, whereas adaptations in cardiac response determine CVP and normally prevent a further rise as exercise continues.

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CHAPTER 5

Exercise Capacity and Energy Expenditure of Morbidly Obese and Previously Obese Subjects

Chapter Link

Cardiac output is matched to the actual oxygen cost of exercise while heart rate increases in proportion to the relative oxygen consumption or per cent of maximum during dynamic exercise. The former holds true even in the case of cardiac denervation as shown in chapter 3. In the next study, I tested whether heart rate remained a function of relative effort even when the metabolic response to a given work rate was markedly altered. This occurs when individuals undergo a large rapid change in body mass which can be induced by isolated gastric bypass surgery. This patient group provided a model for studying the heart rate/oxygen consumption relationship when oxygen consumption at a given level of work was markedly different, due solely to body mass changes. I assumed that lean body tissue would be maintained after weight loss and peak absolute oxygen consumption would be similar in obese and previously obese groups provided normalized energy expenditure was similar. Unexpectedly, peak oxygen consumption was not maintained after normalization of body weight at one year post surgery, however, this was not evident at six months post surgery in a longitudinal group.

A. ABSTRACT

Morbidly obese and previously obese women were studied to determine if: 1) failure to lose weight after surgery is due to a low activity level; 2) large surgically-induced rapid weight loss improves exercise capacity. Four groups of 5 subjects were studied in a cross-sectional design. These included: 1) patients still obese 12 months after surgery (failure); 2) patients who normalized their weight post surgery (success); 3) preoperative obese patients (preop); and 4) non-obese subjects, (control). Four of the preop group were studied again at 6 months post-surgery. Peak oxygen consumption ($\dot{V}O_2$) provided a measure of exercise capacity. Total daily energy expenditure (TDEE) was calculated using the flex heart rate method of Ceesay et.al.(1989). TDEE or energy expenditure (EE) above basal metabolic rate when normalized for body size were similar among groups. However, peak absolute VO_2 was decreased in the success group ($p<.05$). Peak $\dot{V}O_2$/kg was similar in the failure, preop and success groups and all were lower than control ($p<.05$). The heart rate was higher at a given absolute $\dot{V}O_2$ in the success compared with the failure and preop groups ($p<.05$) consistent with a decreased aerobic capacity in the success group, possibly from loss of muscle mass. This was not evident at 6 months post-surgery in the longitudinal group. Failure to lose weight following isolated gastric bypass surgery was not associated with a decreased activity level. Aerobic capacity was impaired

one year, but not 6 months, after a large weight loss. Exercise training may be appropriate to maintain absolute peak oxygen consumption.

B. INTRODUCTION

Morbidly obese patients have a high risk for disease, therefore drastic measures are sometimes undertaken to induce weight loss (8,18). This includes the isolated gastric bypass procedure which has been successful in normalizing body weight in 83% of patients within 12 to 18 months (22). However, some patients still fail to lose weight following technically acceptable surgery. This may result from a decrease weight-adjusted energy expenditure due to lower activity level and/or a lower resting metabolic rate in failed vs successful outcomes. Alternatively, failed outcomes may continue to maintain an increased energy intake despite restrictive surgery, although this is difficult to assess under free-living conditions. We can identify which factor accounts for failure to lose weight following surgery by assessing energy expenditure in failed and successful surgical outcomes compared with non-surgical obese and lean subjects.

Furthermore, in the case of successful surgical outcomes, the affect of a large and rapid weight loss on aerobic function has not been systematically addressed. Since obese subjects carry extra weight but generally have a normal weight-adjusted daily energy expenditure (14,25), their total energy expenditure is increased compared with lean subjects. Whether they maintain their aerobic capacity following weight loss and thereby increase external work capacity is not clear. We can study this both cross-sectionally, by comparing peak

oxygen consumption of failed and successful surgical patients and non-surgical obese and lean subjects, and serially by comparing responses pre and post surgery in the same individuals.

Previous work has shown that absolute peak oxygen consumption ($\dot{V}O2$) is similar in moderately obese and lean controls during both weight supported and weight bearing exercise but $\dot{V}O2$ is elevated at a given work rate in obese subjects (12,28,29), presumably because of work involved in dealing with the extra weight and adipose tissue. Therefore, obese individuals have a reduced work capacity and this might be expected to improve with weight loss. However, in one study in which morbidly obese subjects were examined before and two years after jejunoiliostomy-induced weight loss, the heart rate at two submaximal oxygen consumption levels was increased after weight loss was complete. This suggests that the relative load was higher in the previously obese after weight loss, indicating a decrease in peak $\dot{V}O2$ although this was not assessed (5).

We therefore studied morbidly obese, previously obese and non-obese women to determine if: 1) failure to lose weight after surgery is due to a low activity level; 2) large, rapid weight loss, due to surgical intervention, results in improved exercise capacity.

C. METHODS

Subjects: The study was approved by the hospital committee for human experimentation and all subjects gave informed consent. We studied 20 females; 5 were normal weight, had never been obese and served as control (control); 5 were morbidly obese and were awaiting isolated gastric bypass surgery (preop); 5 were still morbidly obese one year after technically acceptable isolated gastric bypass surgery (failure); 5 had been morbidly obese subjects but normalized their body weight one year after surgery (success). Technically successful surgery was assessed by follow-up endoscopy examination. Morbid obesity was defined as a body mass index (BMI) of greater than 40 kg/m^2. Normal weight referred to a BMI of less than 25 kg/m^2. Four of the preoperative group were re-evaluated six months following surgery.

All obese patients were otherwise healthy, non-diabetic and free from metabolic disorders and medications with the exception of one patient in the failure group who was taking a diuretic and one in the preoperative group who was on methyldopa for treatment of hypertension. Subject characteristics are described in Table 5.1.

Energy Expenditure: We calculated total daily energy expenditure (TDEE) in kilojoules (kj) minute by minute for 24 hours from the heart rate (HR) and the "flex HR" analysis (10,21). The subjects wore a small, light electrode belt transmitter and a wrist microcomputer receiver which had a

TABLE 5.1. Description of subjects who failed to lose weight following gastric bypass surgery for obesity (failure); successfully lost weight following surgery (success); were pre-operative (pre-op); were non-obese control subjects (control). yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; kg/m^2, kilograms per square metre; HR, heart rate; bt/min, beats per minute; $\dot{V}O2$, oxygen consumption. Mean ± Standard Error. *, p<.05.

Mean±S.E.	FAILURE	SUCCESS	PRE-OP	CONTROL
n	5	5	5	5
AGE (yrs)	42.6 ± 2.8	33.0 ± 3.2	39.8 ± 4.6	34.6 ± 2.0
HEIGHT(cm)	160.5± 3.6	161.1± 3.9	161.0± 1.7	167.7± 2.9
WEIGHT(kg)	102.7± 8.2*	61.6 ± 2.2	132.4± 4.4*	57.7 ± 1.6
PRE-OP WEIGHT(kg)	106.4± 8.2	116.5± 4.0	----------	----------
BMI(kg/m^2)	39.7 ± 1.4*	23.8 ± 0.6	51.0 ± 1.7*	20.6 ± 0.4
Rest HR (bt/min)	74.8 ± 4.2	75.0 ± 4.0	84.0 ± 5.7	75.8 ± 4.2
PEAK HR	172.2± 5.8*	163.0± 4.6*	159.6± 3.2*	186.6± 3.6
Rest $\dot{V}O2$ (ml/min)	250.0 ± 18.5*	185.8± 17.1	274.0 ± 22.5*	194.0± 7.2
PEAK $\dot{V}O2$ (ml/min)	2370.6 ± 309.4	1312.6 ± 162.2*	2339.4 ± 303.5	2714.0 ± 270.7
PEAK $\dot{V}O2$ (ml/kg min)	22.8 ± 1.4*	21.4 ± 2.8*	17.6 ± 2.1*	47.2 ± 4.9

total minute by minute data storage capacity of 33 hours (Polar™ Vantage XL®, Model #145900, Polar CIC Inc.,Port Washington, NY). HR for each minute was retrieved by connecting the wrist unit to a computer interface and downloading the data to computer. Flex HR was defined as the mean difference between the lowest exercise HR on the treadmill protocol and the highest HR achieved during a series of rest or light activities including sitting quietly, changing position to standing, standing quietly on the treadmill and beginning to walk. This distinguishes between periods of low activity, where the $\dot{V}O_2$/HR relationship is weak, and periods of higher activity where there is a strong linear relationship between the two variables (4). $\dot{V}O_2$/HR regression equations were calculated for each subject from the graded exercise test and used to calculate $\dot{V}O_2$ from HR for rates above the "flex HR". Energy expenditure for HR's below the individual flex HR were estimated to be the mean resting $\dot{V}O_2$ ($R\dot{V}O_2$), measured from the series of rest or light activities. The caloric equivalent for oxygen was assumed to be 5 kcal/L or 20.93 kj/L. Sleep time was noted for each subject during the monitoring period and energy expenditure during this period (sleep EE)was assumed to be equal to basal metabolic rate (BMR), calculated from the standard equations of Schofield (31). Therefore, the minute-by-minute energy expenditure in kj/min during awake hours was equal to:

$(m \cdot HR+b) \cdot 20.93$ if HR > flex HR; or $R\dot{V}O_2$ if HR < flex HR

where m is the slope and b is the intercept of the activity regression line. These minute values were totalled and added to the calculated sleep EE to generate TDEE for each subject.

This method has been cross validated with indirect whole-body calorimetry by Ceesay et.al. (10) with favourable results (r=.943, standard error of the estimate=458 kj, n=20). It has also been compared favourably with the doubly labelled water technique (21). The coefficient of variation of the BMR calculation has been reported as 8 per cent (31).

Exercise Testing: Subjects performed graded treadmill exercise to a maximal effort. Obese and previously obese subjects exercised at a speed of 2.0 mph with increases in grade of 2.5% every 2 minutes. Control subjects underwent the more progressive Bruce protocol in order to achieve peak effort in less than 15 minutes exercise time. Peak effort was defined as an inability to maintain exercise at the set treadmill speed and a respiratory exchange ratio of greater than 1.0. Heart rate was measured from the 12-lead electrocardiogram at rest and during each minute of exercise and recovery. We obtained expired gases breath by breath to measure oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) at rest and every 30 seconds during exercise and recovery with a computerized metabolic unit (Medgraphics™ CPX, Medical Graphics Corp., Minniapolis, MN). Subjects breathed through a low resistance one-way valve which was connected to the unit via a pneumotachometer. Ventilatory parameters were also measured at

rest and every 30 seconds during exercise and recovery.

Longitudinal Study: We restudied 4 of the 5 pre-op subjects at 6 months following surgery in order to compare with cross-sectional findings.

Statistical Analysis:

Data across groups were analyzed by means of a one-way analysis of variance. Main effects were considered statistically significant at the 95% confidence level and post-hoc Newman-Keuls tests were applied to assess differences between means. We analyzed the longitudinal data by paired t-test. All data are reported as mean ± standard error.

D. RESULTS

CROSS-SECTIONAL STUDY:

Subject Characteristics: Mean age and height were similar across groups. There was no difference in body weight or body mass index between the success and control groups. The preop and failure groups by definition had a significantly higher body weight and body mass index than the other groups ($p<.05$) and values for the preop group were significantly greater than the failure group ($p<.05$). There was no difference between pre-operative body weights of the failure and success groups. Mean weight loss pre to post-surgery was 54.85±3.34 kg ($p<.0001$) in the success group compared with 3.62±4.29 kg in the failure group (N.S.).

Resting oxygen consumption was significantly higher in the

preop and failure groups compared with the success and control groups ($p<.05$) and there was no difference in resting oxygen consumption between the success and control groups. Group means and standard errors are given in Table 5.1.

Energy Expenditure: Total daily energy expenditure (TDEE) was higher in the preop and failure groups vs the success and control groups and this difference persisted when calculated basal metabolic rate (BMR) was subtracted (ExEE). However energy expenditure was similar across groups when normalized for body size using body surface area (Figure 5.1), even when corrected for BMR.

Exercise Capacity: Absolute peak oxygen consumption (Peak $\dot{V}O2$) was decreased in the success group compared with the other groups ($p<.05$). Therefore, the aerobic capacity of the success group was lower than the preop and failed surgical group. Peak VO2 per kilogram of body weight was similar in the preop, failure and success groups and all were significantly lower than controls (Table 5.1).

There was no difference in resting heart rate between groups. Peak heart rate was significantly lower in the preop, failure and success groups than controls ($p<.05$) (Table 5.1). The heart rate/$\dot{V}O2$ per kg relationship during graded treadmill exercise is shown in Figure 5.2A. As metabolic rate increased with increasing work rate, the heart rate increase was similar in the success group and the preop and failure groups whereas the rise was slower in controls which is typical of the

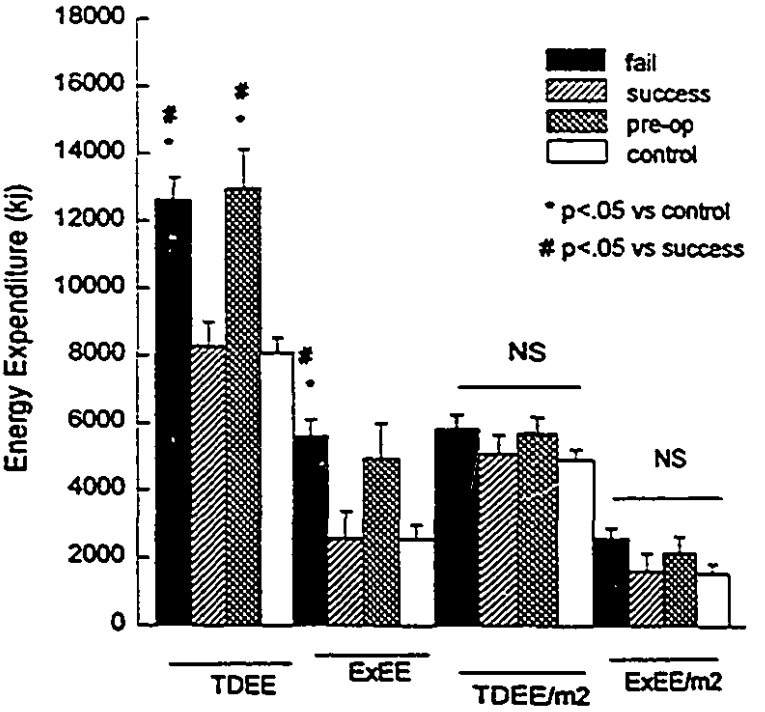

FIGURE 5.1. Energy Expenditure in kilojoules (kj) in four subject groups (n=5 each) plotted as Total Daily Energy Expenditure (TDEE); Exercise Energy Expenditure (ExEE) = (TDEE - Basal Metabolic Rate); TDEE normalized for body surface area (TDEE/m^2); ExEE normalized for body surface area (ExEE/m^2). *Solid bars*, obese patients who failed to lose weight following surgery (fail); *Striped bars*, obese patients who successfully lost weight following surgery (success); *Cross-hatched bars*, pre-operative obese patients; *Open bars*, control non-obese subjects. Mean ± Standard Error. *, p<.05 compared with control subjects; #, p<.05 compared with success group; N.S., not significant.

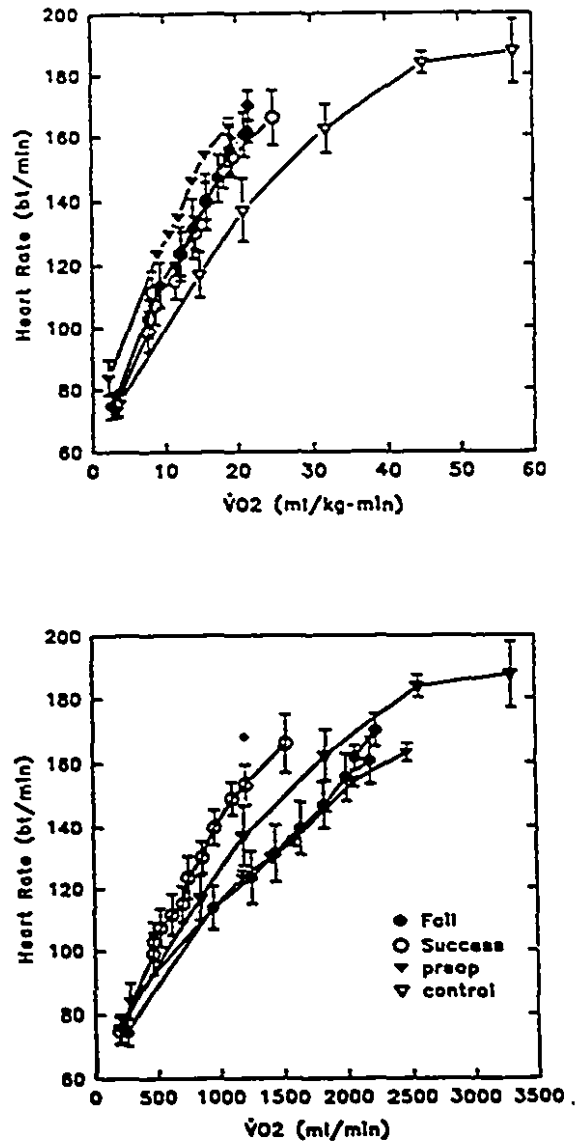

FIGURE 5.2 A. Heart rate in beats per minute (bt/min) plotted against oxygen consumption ($\dot{V}O2$) normalized for body weight in ml/kg•min, in failure group (solid circles), success group (open circles), preop group (solid triangles) and control group (open triangles) during graded treadmill exercise. **B.** Heart rate plotted against absolute oxygen consumption ($\dot{V}O2$) in ml/min during graded treadmill exercise. Mean ± Standard error. *, $p<.05$. Abbreviations as in Figure 5.2A.

trained response, supported by their higher peak $\dot{V}O_2$/kg.

When heart rate is plotted against absolute $\dot{V}O_2$ (Figure 5.2B), the heart rate was higher at a given submaximal $\dot{V}O_2$ (approximately 1250 ml/min) in the success group. This resulted from decreased maximal absolute $\dot{V}O_2$ compared with the other groups and indicates that this submaximal metabolic load demands more effort in these subjects since it represents a higher proportion of their peak oxygen consumption.

Heart rate responses were similar in relation to relative $\dot{V}O_2$ (%$\dot{V}O_2$ peak), with the exception of the controls who reached a higher peak heart rate (Figure 5.3). This indicates similar heart rate control among the different groups.

LONGITUDINAL STUDY:

One subject was lost to follow-up due to relocation. In the remaining 4 subjects, mean weight loss was 41.9±4.74 kg at 6 months post-surgery and mean BMI was reduced from 51.25±2.21 to 34.9±1.41. Thus the subjects were still moderately obese at 6 months post surgery.

Exercise Capacity: There was no statistically significant decrease in absolute peak $\dot{V}O_2$ by 6 months post-surgery (2288±385.7 vs 2083.67±298.4 ml/min). As a result, peak $\dot{V}O_2$ per kg was significantly increased from 17.18±2.7 to 21.8±1.59 ml/kg·min ($p<.03$). The heart rate/absolute $\dot{V}O_2$ relationship (Figure 5.4A) was similar pre and 6 months post-surgery and when $\dot{V}O_2$ was normalized for body weight, heart rate was lower at a given $\dot{V}O_2$ (Figure 5.4B). This is in contrast to the

cross-sectional result where the success group had a decreased absolute peak $\dot{V}O2$ at 12 months post-surgery, at which time they had achieved a normal body mass index.

A decrease in heart rate and absolute $\dot{V}O2$ at a given treadmill work rate occurred with substantial weight loss in the longitudinal study and external work capacity showed a marked improvement as hypothesized (n=4)(Figure 5.5).

E. DISCUSSION

The main findings of this study are that: 1) patients who fail to lose weight following isolated gastric bypass surgery do not have a reduced energy expenditure, when normalized for body size; 2) patients who undergo a rapid and substantial weight loss which normalizes their body weight by one year post-surgery, have a similar peak exercise capacity when expressed as $\dot{V}O2$/kg but a reduced absolute peak $\dot{V}O2$ compared with morbidly obese individuals. This detraining-like effect is not evident during the first 6 months of weight loss.

Before addressing the implications of these findings, we must comment on potential limiting factors in this study.

Limitations: Total daily energy expenditure and basal metabolic rate were calculated rather than directly measured in this study. The flex heart rate analysis has been well validated with more direct techniques of energy expenditure (10,21) and made assessment of these patients possible under free-living conditions with little interference. Our analysis

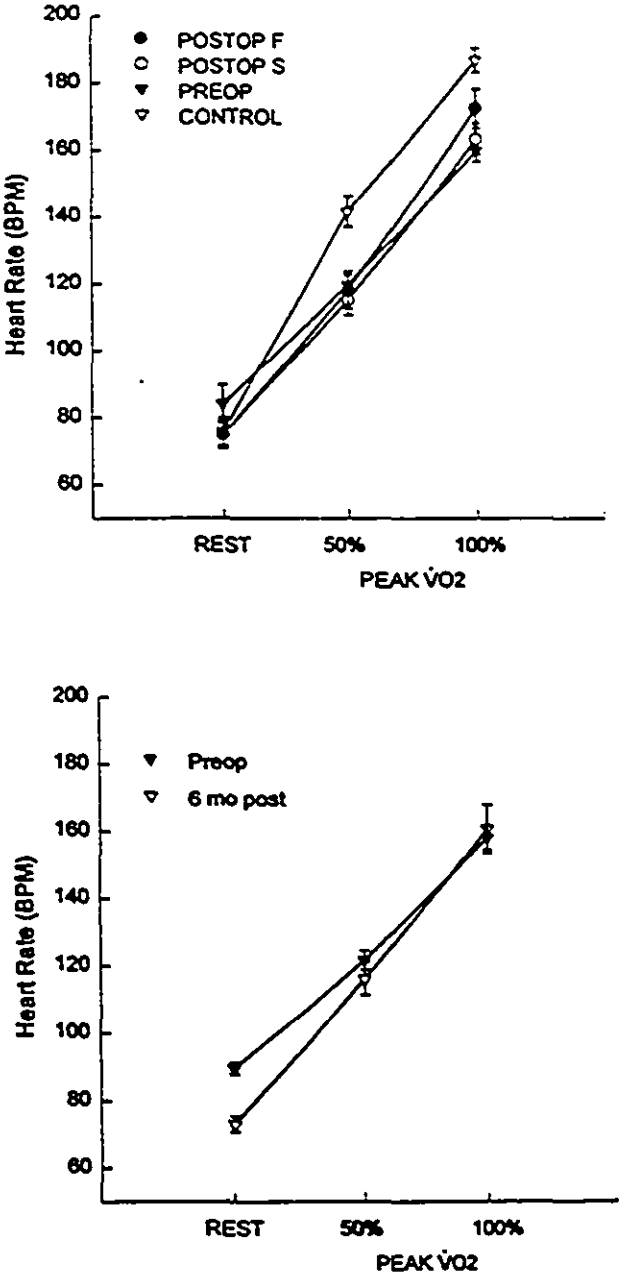

FIGURE 5.3. Heart rate plotted against relative V̇O2 (%V̇O2peak) in the four subject groups (*upper graph*) and longitudinal group (*lower graph*). Mean ± Standard error. Abbreviations as in Figure 5.2.

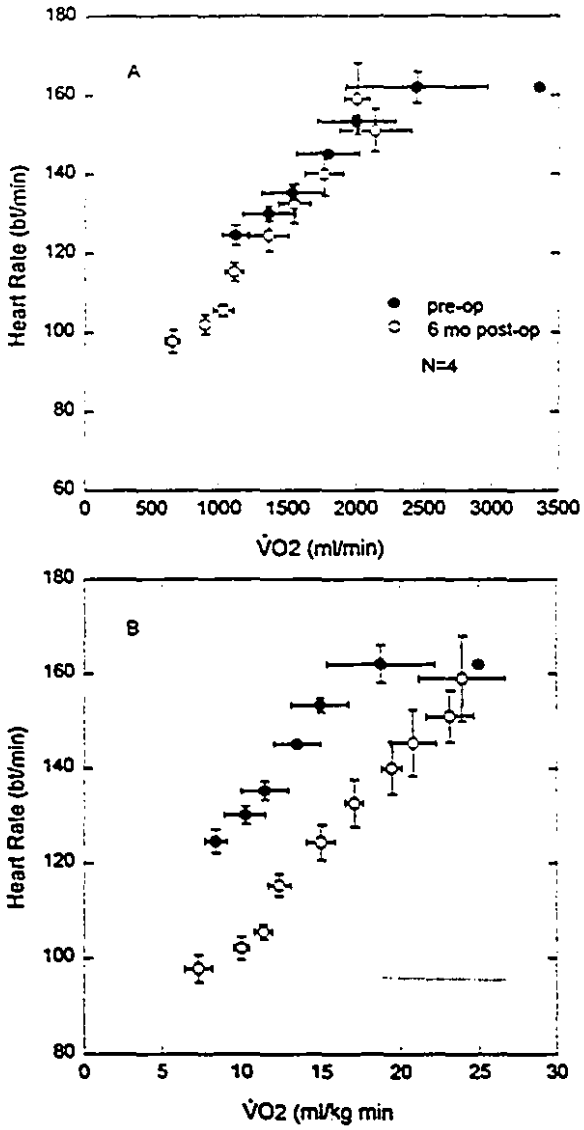

FIGURE 5.4 A. Longitudinal Study. Heart rate/absolute V̇O2 relationship during graded treadmill exercise in four subjects before surgery (pre-op)(solid circles) and six months following gastric bypass surgery for obesity (6 mo post-op)(open circles). **B.** Heart rate plotted against V̇O2, normalized for body weight, in same pre-op and post-op groups. Mean ± Standard error.

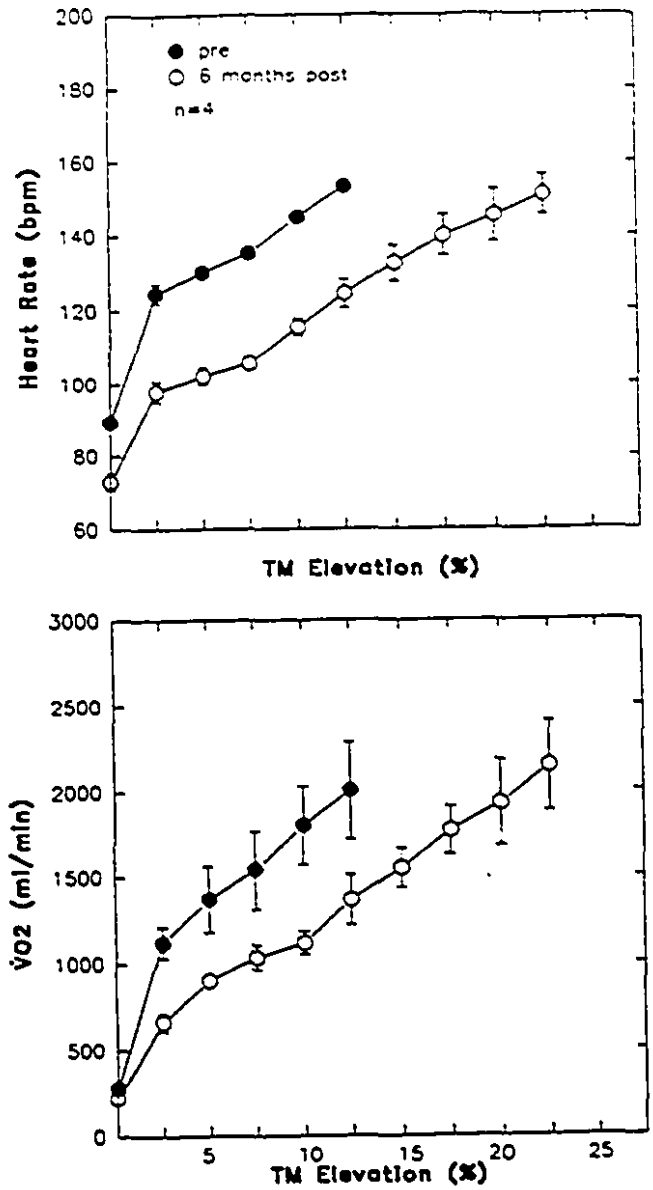

FIGURE 5.5. Heart rate and absolute V̇O2 in relation to treadmill (TM) elevation in percent grade (%) in pre-operative obese subjects (pre)(solid circles) and six months following surgery (6 months post)(open circles). Mean ± Standard error, n=4.

was based on individual rather than group calibration curves and thus gave a more accurate estimate of energy expenditure from heart rate recordings above the flex heart rate value (20). This directly addresses previous concerns regarding the unsuitability of using the heart rate/oxygen consumption relationship to calculate energy expenditure during periods of low activity (11) which is especially important considering the change in patients with weight loss. The values we obtained for total daily energy expenditure in the obese and non-obese groups are comparable to other reports in the literature in which both indirect calorimetry and doubly labelled water methods were used in subjects of similar body mass index (2,9,10,14,15,25,26).

Another potential limitation is the relatively small sample size in each group which could increase the chance of a type II statistical error. However, the flex heart rate method has also been shown to closely estimate energy expenditure in small groups of subjects (33). Also, when the hypothesis of a lower normalized energy expenditure in the failure vs success groups was tested, we found no difference. Indeed, the data showed a slight tendency in the opposite direction. Thus we are confident that rejection of this hypothesis is justified.

Energy Expenditure: Our observation that failure to lose weight following successful gastric bypass surgery is not due to a low energy expenditure fits with previous observations made in moderate to severely obese individuals who

successfully and unsuccessfully achieved a diet-induced weight loss (2). The successful group had a weight loss comparable to our surgical success group. Conversely, some authors have reported decreased energy expenditure in previously obese individuals compared with non-obese control subjects (15,32). However we and others have found no difference (2,9,25). The discrepancy could be the result of a comparatively low food intake which may result in a decreased activity level as well as a decreased thermal effect of food in studies showing a reduced energy expenditure in the previously obese.

Total daily energy expenditure is known to decrease with decreases in body weight during weight reduction but the extent that is due to a reduction in resting metabolic rate or the decrease in energy cost required to support and move body mass is not known (7,14,23,26,34,36). Weigle (36) evaluated the contribution of decreased energy cost of physical activity in men, during weight loss, by exactly replacing the amount of weight lost with a weighted vest which was worn by the experimental group throughout the 3 month diet period. They found that loss of body weight reduced the energy cost of physical activity enough to account for more than half of the fall in energy expenditure, which suggests that changes in the thermic effect of exercise may play a significant role. However, weight replacement did not prevent the fall in fat free mass and the level of physical activity also decreased as weight decreased.

A reduced activity level, expressed as average daily metabolic rate normalized for basal metabolic rate, was also reported in patients after vertical banded gastroplastic surgery and the greatest fat loss occurred in those who had the lowest level of activity (37).

We found no difference in both total and above basal energy expenditure in failure patients compared with preop, success and control groups when normalized for body size. This indicates that their activity pattern was not different and decreased energy expenditure due to decreased physical activity does not explain their failure to lose weight. The question remains why they failed to lose weight following technically acceptable gastric bypass procedure. Thus, the most likely reason is that these subjects maintained their energy intake, in spite of their restricted capacity, either by eating more frequently or making high calorie food choices.

Exercise Capacity: Peak $\dot{V}O_2$ was lower in the previously obese subjects who successfully normalized their body weight one year after gastric bypass surgery, compared with the failed surgical and preoperative groups as well as lean controls. In contrast, Weigle reported no change in maximal oxygen consumption in obese men who underwent a similar weight loss induced by diet (36). It is possible that surgically-induced weight loss in our study induces a more rapid and large weight reduction which accounts for the decrease in peak $\dot{V}O_2$. In the patients studied longitudinally, peak absolute oxygen

consumption did not change six months after surgery, before weight loss was complete. Therefore, the effect of a drastic reduction in energy intake on this variable occurred later in the weight loss period, after prolonged energy restriction. This consequence of surgically-induced rapid weight loss has not been reported previously, although submaximal heart rate data in subjects pre and post jejunoiliostomy have suggested a higher relative load in reduced obese patients at two years post surgery (5).

The most likely explanation for the decrease in peak absolute oxygen consumption in the successful weight loss group is a decrease in fat free mass. Oxygen consumption is strongly associated with fat free mass (24) and thus a significant reduction in fat free mass, particularly muscle mass, would explain the reduced exercise capacity of the successful weight loss group in the present study and their increased relative work load at a given submaximal oxygen consumption. Possible pulmonary function and obesity-induced cardiovascular abnormalities in morbidly obese patients have been shown to improve following weight normalization (1,16,17,27,38).

Decreased muscle mass could result from detraining due to decreased energy expenditure as weight is lost which decreases the intensity of a given activity in reduced obese subjects (27). However, exogenous weight replacement did not reverse the loss in fat free mass in Weigle's study (36), which argues

against a detraining effect. An alternative explanation is that severely restricted food intake may result in a starvation-like burning of protein and an adaptive decrease in oxygen consumption, energy expenditure and fat free mass (3,19,23). This is more likely in our surgical population and may explain why a decrease in peak $\dot{V}O2$ was not observed in diet-induced weight loss studies.

Clinical Implications: Exercise intervention during weight loss can counteract the negative influence of a substantial reduction in weight on exercise capacity and muscle mass (30,35). Exercise training has been shown to reverse most of the decrease in energy expenditure due to reduced resting metabolic rate during weight loss, which would favour long term weight maintenance (34). In addition, a recent study has demonstrated that even during periods of energy restriction which results in large scale weight loss, muscle hypertrophy is possible with resistance training (13). This type of training preserves or increases fat free mass while facilitating body fat loss (6). Therefore, exercise training may counteract decreases in fat-free mass, particularly muscle mass, which may prevent the decreased peak oxygen consumption seen in subjects who have had a large surgically-induced weight loss.

Conclusion: In conclusion, failure to lose weight following isolated gastric bypass surgery was not associated with a decreased activity level. Aerobic capacity was impaired one

year, but not 6 months, after a large weight loss due to energy restriction. Exercise training may be appropriate to maintain fat free mass and preserve absolute peak oxygen consumption as weight loss nears completion.

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CHAPTER 6

Venous Mechanics During Simulated Exercise: Effect of Muscle Contractions

Chapter Link

In the previous chapters I have addressed neurohumoral interactions affecting blood flow distribution and venous return, cardiac function during exercise, the interaction of the heart and the peripheral circuit and mechanical influences at exercise onset and the control of heart rate. What remains to be studied are the specific changes in venous parameters during exercise and their mechanisms of control. In the next study I examined how venous mechanics alter to increase venous return and cardiac output during dynamic exercise. Also, I investigated the contribution of reflex neural effects mediated by thin fibre muscle afferents and the mechanical effect of muscle contraction on these variables. For these measurements it was necessary to use an anesthetized animal model where dynamic exercise could be simulated by intermittent muscle stimulation, invasive measurements of venous parameters and an effective block of afferent nerve traffic from the exercising muscles could be made.

A. ABSTRACT

Alterations in venous capacitance (Vo), resistance (Rv) and compliance (Cv) must contribute to changes in venous return and therefore cardiac output during exercise stress. The goal of this project was to study changes in venous mechanics and cardiac output from 1) activation of muscle afferents with intermittent bilateral sciatic nerve stimulation; 2) muscle contraction alone, by blocking muscle afferent-mediated reflex effects during the stimulation protocol. We studied 2 groups of 6 chloralose-anesthetized and splenectomized dogs during 15 minutes of muscle stimulation (EX); with and without a cold nerve block. Mean circulatory filling pressure (MCFP) was measured by transiently occluding the right atrium with a balloon at baseline and during 2 acute changes in blood volume to assess Cv. Cardiac output (CO) and right atrial pressure (Pra) were measured with a central catheter and Rv calculated from (MCFP-Pra)/CO. CO, heart rate ($p<.05$) and arterial pressure ($p<.01$) increased significantly during EX but not during EX + Block. Rv and Cv did not change. MCFP increased to a similar extent in both EX and EX + block groups but was only significant in the former ($p<.05$). The pressure gradient for venous return (i.e. Pms-Pra) increased during EX ($p<.05$) with no change during EX + Block. We conclude that the pressure gradient for venous return is increased, in this model of light exercise, as a result of changes in cardiac function mediated by muscle afferent stimulation and a possible mechanical efect of muscle contraction on MCFP.

B. INTRODUCTION

The increase in cardiac output during dynamic exercise must involve changes in both heart and peripheral circuit parameters since they are mechanically coupled (9). Yet, little attention has been given to exercise-induced alterations in peripheral vascular factors of the venous system which control venous return and therefore cardiac output in the steady state (8,12,15,21,24,27). These factors are venous compliance, stressed vascular volume, resistance to venous return and the distribution of blood flow between high and low compliant regions (10,12,27). Stressed volume and venous compliance determine mean circulatory filling pressure (MCFP) and these circuit factors are included in Guyton's equation for venous return as follows (10):

venous return=(MCFP - right atrial pressure)/venous resistance

Guyton expressed this graphically as a venous return curve with right atrial pressure on the x-axis and flow or venous return on the y-axis. When this is plotted on the same axes as a cardiac function or Starling curve, the intersection point of the two curves is the steady state cardiac output which must equal venous return (10). This emphasizes that cardiac function changes venous return through changes in right atrial pressure. This type of analysis is useful for separating cardiac from peripheral vascular factors during exercise.

Since the veins are "capacitance" vessels and most of the total blood volume is located there, small changes in venous mechanics during exercise stress would have large effects on venous return and cardiac output (8,15,18).

Venous capacitance decreases in animals when adrenergic drugs are administered, although effects on venous resistance are more variable (1,13,17). Carotid sinus hypotension results in a decrease in venous capacitance as well as a decrease in splanchnic venous resistance and redistribution of blood flow (4). Substantial blood flow redistribution also occurs during dynamic exercise.

The signals which mediate the close matching of cardiac output to metabolic demand during dynamic exercise are thought to include a feed forward central command signal at exercise onset and a feedback signal from the exercising muscle via type III and IV afferents, both of which may be influenced by the arterial baroreceptor (16,23,29,30).When afferent feedback from dynamically exercising muscle is blocked, exercise-induced increases in arterial pressure are reversed whereas effects on heart rate and cardiac output are more variable (5,26,28), although the latter is not often measured.

In addition to neural adjustments, the mechanical effect of muscle contraction may help adjust flow during dynamic exercise (22). We have previously showed that muscle contractions decrease the time constant of venous drainage of the isolated canine gastrocnemius (18). Sheriff et.al.(25)

found that the muscle "pump" was more important than reflex adjustments in capacitance in mobilizing volume in response to an induced fall in cardiac output during mild exercise levels. However, the relative contribution of reflex adjustments and mechanical effects of muscle contraction on overall venous pressure-volume relationships has not been directly addressed.

Therefore the aims of this study are: 1) to assess the effects of electrically stimulated dynamic muscle contractions on venous resistance, capacitance and compliance in anesthetized dogs in both supine and head-up tilt postures. 2) to isolate the mechanical pumping effect of muscle contractions on venous mechanics and cardiac output by blocking reflex adjustments mediated by muscle afferents with a cold block of the nerve proximal to the stimulating electrodes.

We hypothesize that this simulated dynamic exercise will result in: 1) decreases in venous capacitance and resistance, from both reflex adjustments and recruitment of volume during muscle contractions; 2) no change or a small decrease in venous compliance due to muscle contraction; 3) a small effect of the muscle pump on changes in venous mechanics and cardiac output during exercise compared with reflex effects mediated by thin fibre muscle afferents which result in sympathetic activation; 4) an greater decrease in venous capacitance during muscle stimulation with the hindlimbs in a dependent posture.

C. METHODS

Surgical Preparation

Seventeen mongrel dogs (16-26 kg) were anesthetized with 20mg/kg of thiopental sodium followed by 80 mg/kg of alpha chloralose, with supplemental doses of the latter given as necessary. Alpha chloralose was used because it does not depress cardiovascular reflexes. Animals were intubated and ventilated. We placed a catheter in the left carotid artery in order to measure arterial pressure and a balloon tipped catheter was inserted via the jugular vein into the right atrium in order to transiently stop blood flow for measurement of mean circulatory filling pressure and for infusion of fluids. A Swan-Ganz catheter was inserted in the other jugular vein and advanced through the right heart into the pulmonary artery in order to measure cardiac output by thermodilution and right atrial or central venous pressure, pulmonary artery and capillary wedge pressures. A branch of the femoral vein was isolated and cannulated and a catheter advanced to the inferior vena cava in order to obtain a second measure of central venous pressure. We placed a catheter in the left brachial artery in order to infuse the volume loads for the compliance measurement. A ventral laparotomy was performed and the spleen isolated. The spleen was ligated after contraction was induced by injection of 2 mg of epinephrine into the splenic artery and the incision sutured. In some animals (n=5) an electromagnetic flow probe (Carolina Medical Electronics

Inc., King, N.Carolina, U.S.A.) was placed around the terminal aorta proximal to the femoral bifurcation in order to measure flow to both hindlimbs. Right and left sciatic nerve trunks that innervate hamstring, gastrocnemius and foot extensor muscles were isolated and stimulation electrodes attached. The distal ends of the femurs were fixed with steel pins to a steel frame so that movements of the knee and ankle were performed with the hip joint fixed at 90 degrees.

Measurements

Blood Volume:

We used a dye-dilution technique to measure blood volume (2). Two ml of a 0.0017% dilution of Evan's blue dye was injected into the femoral vein and the catheter was flushed with 10 ml of saline.

Arterial blood was sampled after 5, 10 and 15 minutes. The blood was centrifuged and absorbance of the serum was measured by a spectrophotometer (Milton Roy Spectronic 1001 Plus) Plasma volume was calculated using the equation:

$$PV = \frac{2 * \text{control absorbance} * \text{dilution factor}}{\text{absorbance of the 15 minute sample}}$$

Blood volume was then calculated as:

$$BV = \frac{PV}{1 - Hct}$$, where Hct = hematocrit.

Changes in plasma volume were determined by a standard formula, using simultaneous measures of hemoglobin and hematocrit at different times during the experiment (14). In this way we could account for changes in blood volume due to filtration or absorption of interstitial fluid.

Mean Circulatory Filling Pressure

An inflatable balloon attached to the tip of a catheter was placed in the right atrium. We measured mean circulatory filling pressure (or mean systemic pressure) by rapidly inflating this balloon to transiently arrest the flow (ventilator is turned off) until the central venous pressure reached a plateau (10-20 seconds). This plateau pressure is the pressure distending the compliant region of the vasculature at zero flow. The mean of the values obtained from the superior and inferior catheters was taken as the measure of mean circulatory filling pressure.

Hemodynamics

We measured cardiac output by thermodilution and recorded pulmonary artery, right atrial, pulmonary wedge and systemic arterial pressure just prior to inflation of the right atrial balloon in each condition. Heart rate was recorded from a five-lead electrocardiogram on-line using a cardiotachometer (Gould Inc.). Pressures were measured with Trantec transducers (American Edwards Laboratory, Irvine CA). The zero pressure reference point was at the level of the right atrium. The signals were amplified with a preamplifier (Hewlett-Packard

8805A) and the outputs were fed into a computer using a data acquisition program (AT-CODAS, version 5.5, Dataq Instruments Inc., Akron, Ohio) as well as recorded on an eight-channel recorder (Hewlett-Packard 7418A).

The zero pressure reference point was anatomically verified at the end of each experiment and pressures were corrected as necessary.

Venous Compliance and Capacitance

Vascular compliance was determined by measuring the mean circulatory filling pressure (as above) after each of two acute changes in volume by rapid infusion of 5 ml/kg and 10 ml/kg of blood which had been previously withdrawn following injection of 10 ml/kg of dextran. After the last measurement of mean circulatory filling pressure, the same volume of blood was immediately withdrawn. The compliance was calculated from the change in volume divided by the change in mean circulatory filling pressure for each animal. Pressure/volume curves were constructed for each dog. Stressed volume was calculated as the product of mean circulatory filling pressure and compliance. Unstressed volume (Vo) or capacitance, the volume of blood below which there is no distension of vessels, was determined by subtracting stressed volume from total blood volume.

Venous Resistance

Venous resistance (Rv) was calculated using Guyton's equation for venous return (below) (10) using the measured

cardiac output (venous return), mean circulatory filling pressure (MCFP) (upstream pressure) and central venous pressure (downstream pressure and indicative of right atrial pressure (Pra)).

$$Rv = \frac{(MCFP - Pra)}{\text{venous return}}$$

Protocol

Following surgery and a stabilization period of 30 minutes, we determined initial plasma volume with Evan's blue. After baseline measures we imposed the following interventions in separate groups of six dogs each: 1) 15 minutes of intermittent bilateral sciatic nerve stimulation; 2) the same stimulation with the nerves cooled to 1°C to block thin fibre muscle afferent transmission (6). The order of measurements during baseline, nerve stimulation and nerve stimulation with cold block were as follows: arterial and mixed venous blood gases, hemoglobin and hematocrit, hemodynamics, right atrial balloon inflation for measurement of mean circulatory filling pressure. The same measures were repeated after infusion of the two known volumes for compliance measurement and subsequent volume withdrawal. During 15 minutes of muscle stimulation, blood gases and hemodynamics were measured beginning at 2 minutes of exercise followed by the 3 measures of mean circulatory filling pressure at 0, 5 and 10 ml/kg

volumes. Measurements were repeated following volume withdrawal. All were completed by the end of the 15 minute nerve stimulation period. The same sequence was followed for baseline and exercise periods following cold blockade of nerves proximal to the stimulation electrodes. Oxygen consumption was calculated as the product of cardiac output and the oxygen content difference of arterial and mixed venous blood.

Electrical Stimulation Parameters

A dynamic pattern of muscle stimulation was induced using intermittent tetanic contractions. The nerves were stimulated bilaterally with 15-30 trains/min., each train lasting 500 msec. and consisting of rectangular pulses. Parameters of stimuli were 2 times bilateral twitch threshold (=6-10 v * 2) with a duration of 0.2 ms and a frequency of 25-30 Hz. This is similar to dynamic patterns used by others (28) and has shown to be effective in producing significant increases in heart rate, arterial pressure and limb blood flow. We calculated work output using a displacement transducer attached by springs to fine steel wires which were connected to cuffs around each ankle by a pulley system. Force was measured by a transducer (Grass Instruments, Quincy, MA) mounted in series with the springs.

Proximal Cold Blockade

Following baseline measures, cooling of the sciatic nerves to a temperature of 1°C was accomplished in 6 animals by

surrounding each nerve with an insulated thermode perfused with cold fluid (28) proximal to the stimulating electrodes. Cooling began 10 minutes prior to stimulation and continued during nerve stimulation in this intervention. Cold block of type III and IV muscle afferents during exercise could be assessed by observing cardiovascular responses.

Postural Change Protocol

Baseline measures were repeated in 5 additional animals in the supine position as above. These animals then underwent a 20 degree head up tilt and baseline and stimulation protocols were repeated. This was done to account for possible differences in responses due to posture. Venous distension in the hindlimbs would be maximized when in a more dependent position during tilt, increasing the amount of volume which could be recruited. The zero reference of the pressure transducers were re-adjusted when the tilt posture was assumed.

Statistical Analysis

Dependent measures were compared within groups (baseline vs stimulation) and between groups (stimulation with and without cold block) by a 2 factor analysis of variance (ANOVA) with repeated measures on one factor. Main effects were considered statistically significant at the 95% confidence level and post-hoc Newman-Keuls tests were applied to assess differences between means. External work and power output during stimulation with and without cold block were compared by

unpaired t-tests. Dependent measures were compared across treatments (supine, tilt, tilt and stimulation) in the postural change protocol by a one-way Anova for repeated measures. All data are reported as mean ± standard error.

D. RESULTS

Total blood volume was measured in 3 animals in the stimulation group and 4 animals in the stimulation with cold nerve block group and was not significantly altered during the experiments or between the two groups. Stimulated dynamic pattern muscle contractions resulted in an increase in oxygen consumption in both groups ($F=47.2, p<.001$). The mean increase was greater in the unblocked group (134%) compared with the blocked group (88%) although it did not reach statistical significance (Table 6.1). Similarly, external work and power output during stimulation were not significantly different between groups.

Cardiovascular Response:

Baseline measures of cardiac output, heart rate, arterial-mixed venous oxygen content difference and oxygen consumption were not different between groups. However, baseline mean arterial pressure was significantly higher in the blocked group compared to the unblocked group ($p<.01$) (Table 6.1).

Cardiac output ($F=17.71, p<.005$), heart rate ($F=35.3, p<.001$) and arterial pressure were significantly increased by muscle stimulation ($p<.01$), and this increase was abolished when

TABLE 6.1 Cardiac and Peripheral Vascular Responses at Baseline and During Muscle Stimulation With and Without Cold Nerve Block. Mean ± S.E; Pra, right atrial pressure; MCFP, mean circulatory filling pressure; Cv, venous compliance; Rv, venous resistance; $\dot{V}O_2$, oxygen consumption; $(a-\bar{v})O_2$, arterial-mixed venous oxygen content difference.

	BASELINE	STIM	BASELINE	STIM + BLOCK
Mean ± S.E.	n=6		n=6	
Body Weight (kg)	21.5 ± 0.8	---------	20.7 ± 1.8	--------
Blood Volume (ml/kg)	85.3 ± 5.1	86.2 ± 6.5	91.3 ± 7.7	94.6 ± 9.5
Pra (mmHg)	3.8 ± 0.5	2.7 ± 0.5	3.5 ± 0.5	3.6 ± 0.6
MCFP or Pms	11.2 ± 0.6	12.1 ± 0.6*	10.9 ± 0.5	11.7 ± 0.6
Cv (ml/kg•mmHg)	2.6 ± 0.5	2.0 ± 0.2	2.7 ± 0.5	2.2 ± 0.2
Rv (mmHg/l•min)	3.0 ± 0.4	2.7 ± 0.3	3.1 ± 0.4	2.9 ± 0.3
Heart rate (bt/min)	140.2 ± 7.0	187.8 ± 9.0 *	150.0 ± 4.7	159.2 ± 5.4
Arterial pressure (mmHg)	124.2 ± 3.7	136.7 ± 3.1 *	131.7 ± 9.1 *	137.5 ± 7.8
Cardiac output (l/min)	2.6 ± 0.3	3.6 ± 0.4 *	2.5 ± 0.3	2.9 ± 0.3
Femoral flow	1.2 ± 0.4 (n=3)	2.3 ± 0.1	0.9 ± 0.6 (n=2)	1.5 ± 0.4
$\dot{V}O_2$ (l/min)	97.5 ± 7.0	228.1 ± 21.4*	104.5 ± 24.6	196.7 ± 37.9*
$(a-\bar{v})O_2$	38.0 ± 2.5	65.3 ±5.3*	38.8 ± 4.9	64.2 ±6.7*
External work (kgm)	----------	0.12 ± 0.05	----------	0.07 ± 0.03
Power output (watts)	----------	0.4 ± 0.1	----------	0.3 ± 0.1

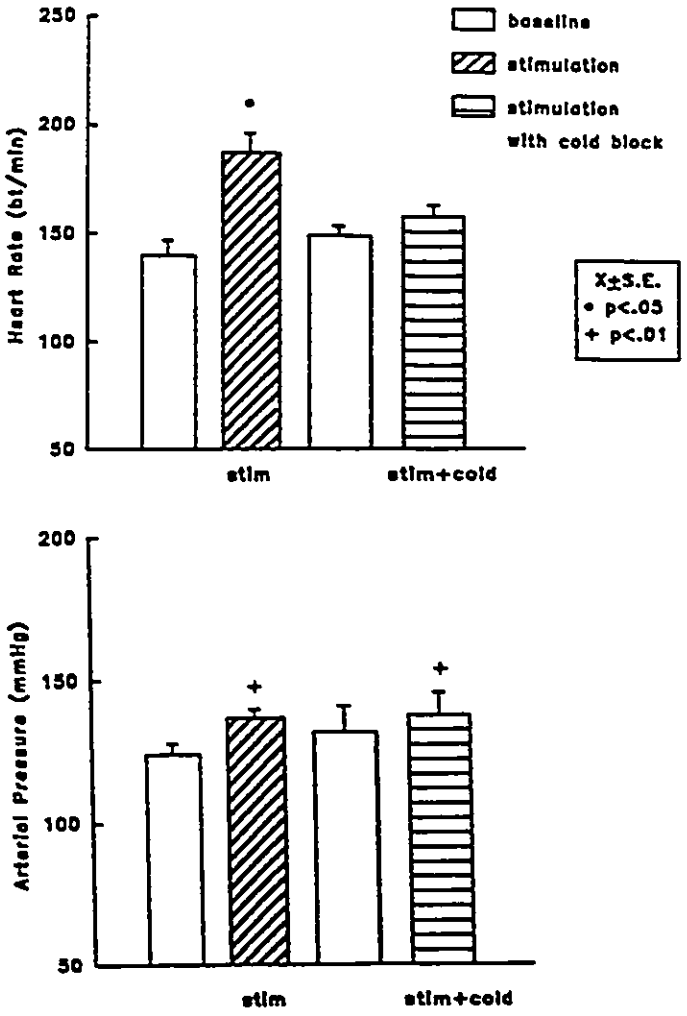

FIGURE 6.1. Heart rate and arterial pressure responses at baseline and during muscle stimulation with nerve intact (stim) and nerve blocked (stim + cold). *Open bars*, baseline measures for both groups; *diagonal striped bars*, muscle stimulation with nerve intact (n=6); *horizontal striped bars*, muscle stimulation with nerve blocked (n=6). Mean ± S.E.; *, p<.05; +, p<.01.

thin-fibre muscle afferent transmission was blocked by cooling the nerve. There was no significant difference between groups in central venous pressure, although it tended to decrease with muscle stimulation in the unblocked group. Mean values and standard errors of the mean are given in Table 6.1. Mean heart rate and arterial pressure at baseline and after 2 minutes of muscle stimulation in both unblocked and blocked groups are shown in Figure 6.1.

Cardiac function curves were constructed by plotting cardiac output against right atrial pressure before and after each level of volume infusion at baseline and during muscle stimulation in both groups (Figure 6.2). Cardiac output increased during muscle stimulation ($p<.05$). There was a shift of the cardiac function curve upward and to the left during muscle stimulation, indicating an increase in cardiac function, which was abolished by blocking thin fibre muscle afferent input.

Peripheral Vascular Response:

There was a main effect of muscle stimulation on mean circulatory filling pressure ($F=7.77, p<.025$) such that it was significantly increased during muscle stimulation with intact nerve ($p<.05$) but not during cold nerve block, although the magnitude of increase was similar. Venous return curves were plotted by joining the pressure in the compliant region at zero flow to the steady state cardiac output and right atrial pressure point, shown in Figure 6.3. Since mean circulatory

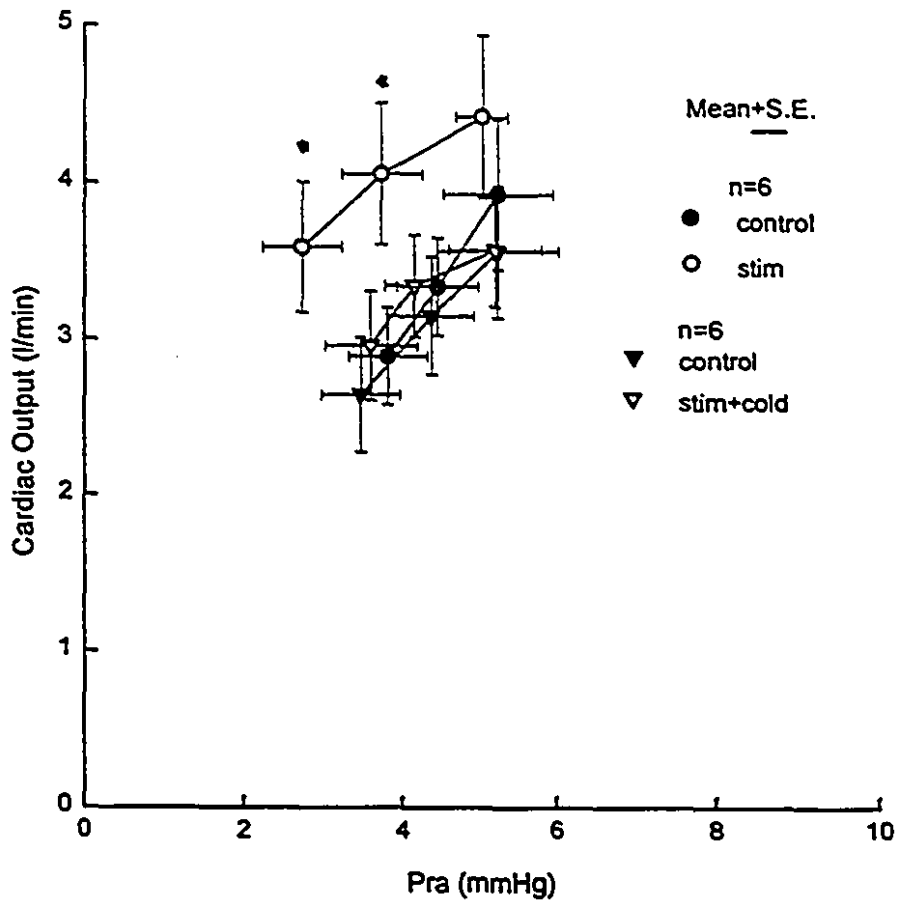

FIGURE 6.2. Cardiac function curves during baseline control (closed circles), during muscle stimulation with nerve intact (open circles) (n=6), and during baseline (closed triangles) and muscle stimulation with nerve blocked (open triangles) (n=6). Mean ± S.E.; *, $p<.05$ compared with baseline. Pra, right atrial pressure.

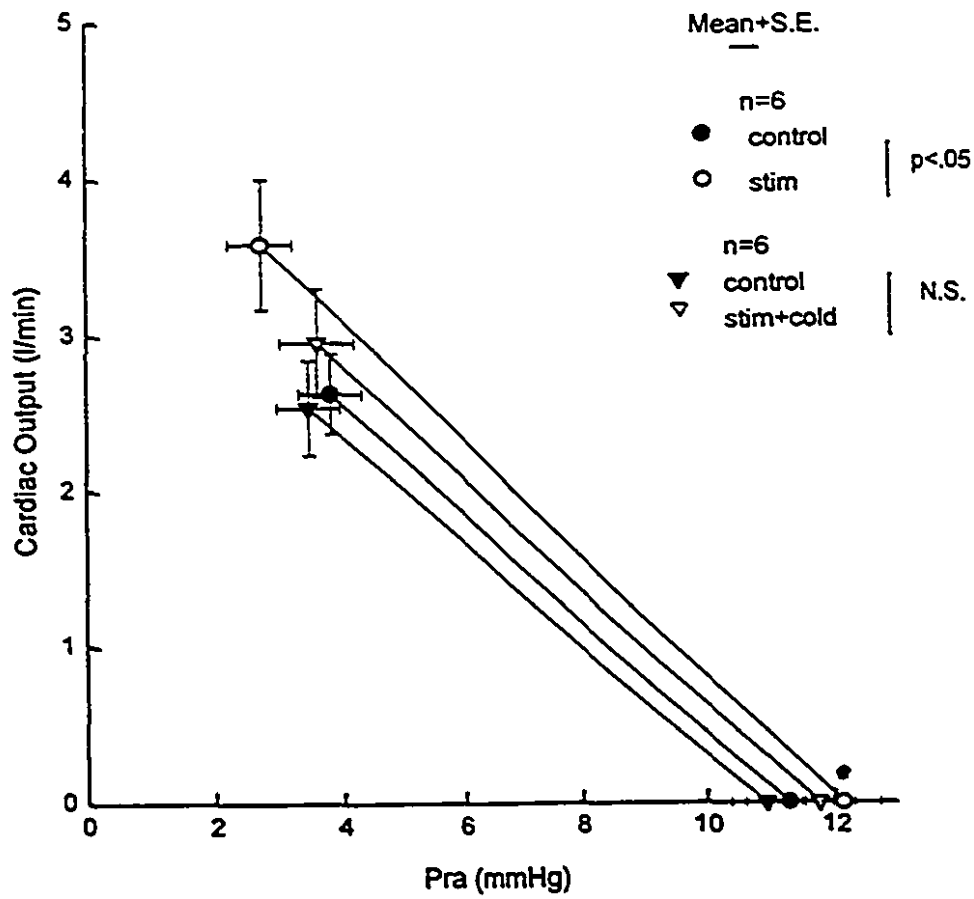

FIGURE 6.3. Venous return curves during baseline control (closed circles), during muscle stimulation with nerve intact (open circles) (n=6), and during baseline (closed triangles) and muscle stimulation with nerve blocked (open triangles) (n=6). Mean ± S.E.; *, p<.05 compared with baseline. Pra, right atrial pressure.

filling pressure is the x-intercept on the venous return curve, the increase during muscle stimulation resulted in a shift in the curve to the right and an increase in the steady state venous return or cardiac output.

Venous compliance and resistance to venous return were not significantly different from baseline or between groups. Due to technical difficulties, we were only able to obtain blood volume measurements in a subgroup of animals in each group. Calculated mean stressed vascular volume was similar during muscle stimulation with (n=4) and without cold block (n=3) and neither were significantly different from baseline, although the variability was high. This indicates that there was no change in venous capacitance, although the number of animals may have been too small to detect a difference.

The mean fractional blood flow to both hindlimbs increased from 44% at baseline to 64% (n=3) during stimulation, and from 35% to 52% (n=2) during the cold block.

Response to Postural Change:

A 20 degree tilt resulted in a decrease in mean cardiac output ($p<.05$), mean arterial pressure ($p<.05$), central venous pressure ($p<.05$) and mean circulatory filling pressure ($p<.05$), and an increase in mean arterial-mixed venous oxygen difference ($p<.05$) compared with supine baseline measures. There was no significant change in mean oxygen consumption, heart rate, venous compliance, venous resistance, venous capacitance or total blood volume.

During muscle stimulation in the tilted position, the increase in oxygen consumption ($p<.05$) was similar to that seen in the previous protocol. With tilt there was an increase in cardiac output and arterial-mixed venous oxygen difference ($p<.05$) but no significant change in heart rate, arterial pressure, mean circulatory filling pressure, central venous pressure, venous compliance, venous resistance and venous capacitance during muscle stimulation while tilted compared with the baseline tilt position. However, venous resistance decreased during muscle stimulation compared with baseline tilt values in all but one animal, which contributes to the change in slope seen in the venous return curve depicted in Figure 6.4. Mean values and standard errors of the mean are given in Table 6.2.

Cardiac function curves during supine, tilt and stimulation during tilt conditions are shown in Figure 6.5 and demonstrate a displacement in the cardiac output operating point to a lower point along the same function curve during tilt and a small shift in the curve to the left as cardiac output increased at the same right atrial pressure during muscle stimulation.

E. DISCUSSION

The main findings of this study are that: 1) heart rate and cardiac output increase in response to reflex adjustments mediated by thin fibre muscle afferents during simulated

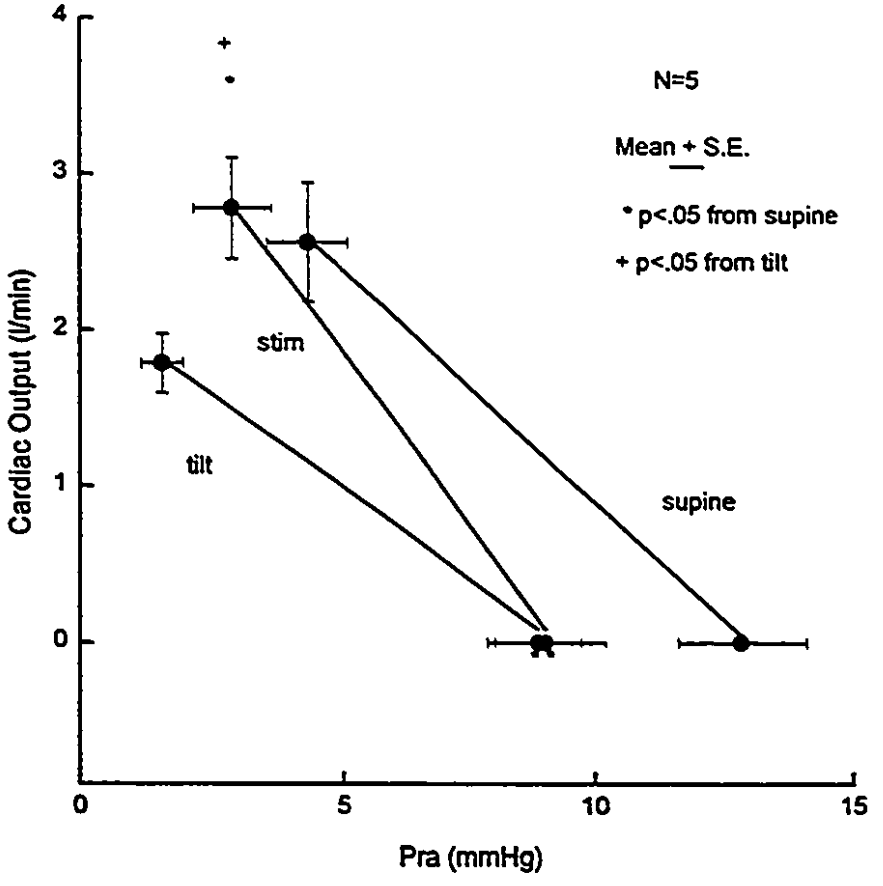

FIGURE 6.4. Venous return curves during supine baseline, (supine), during 20 degree head-up tilt (tilt) and during muscle stimulation while tilted (stim) (n=5). Mean ± S.E.; *, p<.05 compared with supine; +, p<.05 from tilt. Pra, right atrial pressure.

TABLE 6.2 Cardiac and Peripheral Vascular Responses During Head-Up Tilt and Following Muscle Stimulation in the Tilted Posture. Mean ± S.E. (n=5); abbreviations as in Table 6.1.

	SUPINE	TILT	TILT + STIM
Mean ± S.E.	n=5		
Body Weight (kg)	19.6 ± 0.6	---	---
Blood Volume (ml/kg)	94.9 ± 5.5	90.5 ± 6.1	93.7 ± 6.0
Pra (mmHg)	4.3 ± 0.8	1.5 ± 0.4*	2.9 ± 0.7
MCFP or Pms	12.8 ± 1.2	8.9 ± 0.8*	9.0 ± 1.1
Cv (ml/kg•mmHg)	3.0 ± 0.4	4.5 ± 1.0	2.4 ± 0.4
Rv (mmHg/l•min)	3.6 ± 0.5	4.4 ± 0.8	2.3 ± 0.4
Heart rate (bt/min)	140.2 ± 11.5	165.6 ± 17.2	186.8 ± 17.8
Arterial pressure (mmHg)	137.6 ± 6.2	121.0 ±13.4*	120.0 ± 13.5*
Cardiac output (l/min)	2.6 ± 0.4	1.8 ± 0.2*	2.8 ± 0.3 +
$\dot{V}O_2$ (l/min)	102.6 ± 8.1	105.2 ± 11.2	239.0 ±33.1 *
$(a-\bar{v})O_2$	45.5 ± 9.9	64.0 ± 14.4*	85.0 ± 16.0 *
External work (kgm)	---	---	0.13 ± 0.01
Power output (watts)	---	---	0.63 ± 0.4

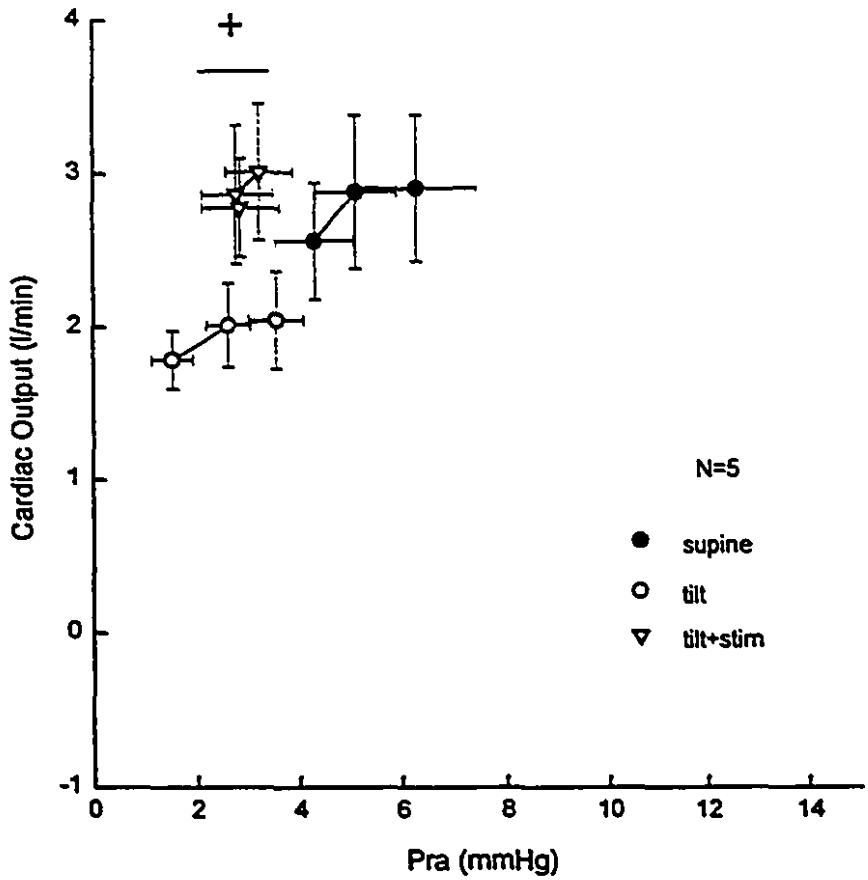

FIGURE 6.5. Cardiac function curves during supine baseline, (solid circles), during 20 degree head-up tilt (open circles) and during muscle stimulation while tilted (open triangles) (n=5). Mean ± S.E.; +, p<.05 from tilt. Pra, right atrial pressure.

dynamic exercise in anesthetized, splenectomized dogs; 2) the pressure gradient for venous return increases through an increase in mean circulatory filling pressure and the tendency for a decrease in right atrial pressure, during dynamic pattern muscle stimulation; 3) there is a possible small mechanical effect of muscle contraction on mean circulatory filling pressure; 4) there was no change in overall venous compliance and resistance during simulated dynamic exercise.

Study Limitations: To assess venous parameters in vivo, it was necessary to use an anesthetized preparation. By stimulating muscle contractions in a dynamic pattern in anesthetized animals, we attempted to simulate dynamic exercise in the conscious animal or human. However, electrically stimulated contractions differ in recruitment pattern and metabolic response from spontaneous voluntary contractions, which may have influenced our results particularly with reference to the mechanical effect of muscle contractions (22). Therefore these results are best compared with other muscle stimulation exercise protocols.

The lack of change in venous mechanics during exercise may be attributed to low intensity of exercise. However, significant increases in cardiac output, heart rate and arterial pressure occured with this protocol which suggests an adequate stimulus. Our overall measures of venous parameters may have been insensitive to significant regional changes.

Cardiovascular Response: Heart rate and arterial pressure

increased as oxygen consumption increased during muscle stimulation as expected. The responses were comparable to those reported by Tibes (28) in a similar preparation. We observed a mean increase in cardiac output of approximately 40% after 2 minutes of muscle stimulation and blood flow to the working muscles in 3 animals doubled. The changes in these variables are consistent with the known cardiovascular response to mild dynamic exercise (20,26).

When both sciatic nerves were cooled to 1 degree C, there was no significant increase in heart rate, arterial pressure or cardiac output during muscle stimulation. The arterio-mixed venous oxygen difference was increased to a similar extent in both blocked and unblocked groups. Mean oxygen consumption was increased during muscle stimulation with nerve block but to a lesser extent than the unblocked group, due to the lower cardiac output.

The lack of change in arterial pressure during stimulation with nerve cooling supports the assumption that a selective block of type III and IV thin fibre muscle afferents was achieved and is in agreement with other studies where the afferent pathway was cut in animals (3) or blocked by epidural anesthesia in humans (5,26). In contrast, Tibes reported an initial decrease in arterial pressure during muscle stimulation with cold nerve block which reverted to baseline by the end of the stimulation period (28). It is evident that thin fibre muscle afferent feedback is necessary for the

increase in arterial pressure during dynamic exercise.

We observed that cold nerve block also prevented the rise in heart rate and cardiac output during muscle stimulation. The lack of heart rate response was also noted by Tibes (28) but contrasts with the normal increase in heart rate observed by Fernandes et.al. during dynamic exercise in humans with epidural anesthesia (5). However, Strange and associates reported a blunted increase in heart rate and cardiac output during electrically stimulated mild dynamic leg extension exercise with epidural anesthesia in humans. They argued that activation of the arterial baroreceptor may have prevented a larger decrease in these variables (26). Activation of the arterial baroreceptor is unlikely in our study since the actual value of arterial pressure was similar in the blocked and unblocked groups during stimulation due to a higher baseline pressure in the blocked group. Therefore our results indicate that feedback from thin fibre muscle afferents is essential for a normal heart rate and cardiac output response to simulated dynamic exercise.

Peripheral Vascular Response: We observed a small but significant increase in mean circulatory filling pressure during muscle stimulation. This pressure is the upstream pressure for venous return and is relatively low under baseline conditions, thus a small increase can have a large impact on venous return (10,13). The downstream or right atrial pressure tended to decrease with muscle stimulation as

a result of increased cardiac function (i.e. heart rate). This further increased the pressure gradient for venous return. The increase in mean circulatory filling pressure (MCFP) could result from either a decrease in venous compliance or a decrease in capacitance (unstressed blood volume) (1,10). However, we found no significant change in either venous compliance or capacitance during muscle stimulation in the present study, although the latter was only assessed in 3 dogs and therefore cannot be ruled out. Resistance to venous return was also unaltered by muscle stimulation. It is possible that our global measures of venous compliance, capacitance and resistance to venous return may not have been sensitive enough to reflect regional changes in fast time constant vs slow time constant beds as has been noted with adrenergic drug administration (1,7,17) and carotid sinus hypotension (4). In addition, changes in overall venous mechanics during dynamic exercise may be more significant at higher work rates where sympathetic outflow is greater.

We observed a parallel shift in the venous return curve during muscle stimulation in both intact and nerve blocked groups. A larger parallel shift in the cardiac output/central venous pressure relationship (equivalent of venous return curve) was reported by Sheriff et.al. in conscious dogs running on a treadmill both with and without autonomic blockade suggesting a role for the muscle pump in increasing venous return by increasing MCFP, although the latter was not

measured directly in that study (25).

Guyton reported a 2 fold increase in MCFP in dogs, during stimulation of the lower spinal cord with maximal tetanizing stimuli, and no change in resistance to venous return (11). The larger MCFP response is likely due to an intact spleen and the different stimulation parameters and method which involved static contractions of the entire lower body musculature including the abdomen. However, the authors could not distinguish between compliance and capacitance effects on MCFP. This stimulation protocol would have cause elevation of the hindlimbs which would shift volume centrally and likely contribute to the elevation in MCFP and cardiac output. The increase in MCFP persisted after autonomic blockade with hexamethonium, suggesting an important role for the mechanical effect of muscle contraction (11).

MCFP was increased during muscle stimulation in the present study both when muscle afferents were intact and blocked with cold nerve block, although only the former was statistically significant. However, the magnitude of change was similar which suggests that in this model of mild dynamic exercise, a mechanical effect of muscle contraction on the downstream pressure for venous return is likely. The lack of change in other venous parameters supports this as does previous work which demonstrates the importance of the muscle pump in redistributing blood volume during exercise (11,19,25). The pressure gradient for venous return was increased during

muscle stimulation with the nerve intact due to a combination of an increased MCFP and a reflex-induced increase in cardiac function. Therefore, at this level of exercise, significant increases in cardiac function were mediated by muscle afferents and increases in MCFP increase venous return and cardiac output. We cannot rule out a contribution by the muscle pump to the increase in MCFP observed. A decrease in capacitance appears more likely to account for the change in MCFP than a decrease in venous compliance since the latter did not change with muscle stimulation.

Postural Change: Contrary to our expectations, recruitment of volume was not enhanced during muscle stimulation in a more upright position, perhaps due to the already increased autonomic tone following the tilt manoeuvre. In spite of this, arterial pressure did not recover completely following tilt and was not increased during muscle stimulation, perhaps due to decreased autonomic reserve. We observed a decrease in venous resistance in 4 of 5 animals during stimulation but no change in mean circulatory filling pressure. This suggests that a decrease in venous resistance may become an important factor for increasing venous return and cardiac output during dynamic exercise under conditions of high sympathetic tone.

In summary, increases in heart rate and cardiac output in this model of simulated mild dynamic exercise occur in response to reflex adjustments mediated by thin fibre muscle afferents. The increase in cardiac output is achieved by an

increase in the pressure gradient for venous return, through an increase in pump function and increase in mean circulatory filling pressure. There is likely also a small mechanical effect of muscle contraction on mean circulatory filling pressure. Overall venous compliance and resistance are unaffected by mild simulated dynamic exercise, however, a decrease in resistance to venous return may contribute to increases in cardiac output under conditions of decreased autonomic reserve.

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CHAPTER 7

General Conclusions

The primary objective of this thesis was to examine the relative importance of neural, humoral and mechanical contributions to the peripheral vascular and cardiac control of cardiac output during the integrated response to exercise stress. In the first study, the influence of the arterial baroreceptors on plasma levels of vasoconstrictor peptides endothelin-1 and neuropeptide Y was examined to determine neural vs local control of these peptides, which have been implicated in the redistribution of blood flow during sympathetic activation. Such neurohumoral interactions may impact on peripheral vascular and cardiac factors controlling cardiac output. Next, peripheral vs cardiac limits to exercise was investigated in heart transplant and normal subjects to determine the relative influence of neurally mediated changes in cardiac function and peripheral vascular factors on cardiac output response and exercise tolerance. The immediate response of central venous pressure at exercise onset in heart transplant and normal subjects was compared in the third study to separate the mechanical effect of muscle contraction on peripheral blood volume mobilization from neurally-mediated changes in cardiac function. Large changes in body mass of morbidly obese patients following surgical treatment was used in the fourth study to investigate whether altering the metabolic signal during exercise would affect cardiac response. In the last study, the effects of simulated dynamic exercise on venous mechanics and cardiac output was studied

and control by muscle afferent neural feedback vs the mechanical effect of muscle contraction in this canine model was determined.

In the first project, I tested the hypothesis that altering carotid sinus input would alter both plasma endothelin-1 and neuropeptide Y whereas inducing systemic hypotension with normal carotid sinus pressure would not. Independent manipulation of carotid sinus and arterial pressure was essential to separate neural and local stimuli because the latter was known to influence endothelin-1 release from vascular endothelium. High carotid sinus pressure decreased plasma levels of neuropeptide Y whereas plasma endothelin-1 was only increased by systemic hypotension in vagotomized animals. This suggests that the carotid sinus can influence plasma neuropeptide Y levels but not endothelin-1 and the increase in endothelin-1 in response to systemic hypotension may be inhibited either directly or indirectly by activation of vagal afferents. This study illustrates the complexity of neurohumoral interactions which can influence blood flow distribution in the intact animal.

In the second project, I tested the hypothesis that cardiac denervation alone does not account for the limited peak oxygen consumption in heart transplant patients. Cardiac vs non-cardiac limits to exercise were assessed by comparing the cardiac output-right atrial pressure relationship of heart transplant patients and normal subjects during graded

exercise, since right atrial pressure reflects the balance of changes in cardiac and peripheral vascular function. Transplant subjects had a steep increase in cardiac output and stroke volume relative to Pra during severe exercise which was similar to normal subjects. This suggests that cardiac function increased until the end of exercise and argues against a cardiac limit in heart transplant patients. Indeed, a drug-induced increase in cardiac function did not increase exercise tolerance in a subgroup of these patients. This study demonstrates the importance of peripheral vs cardiac factors in peak cardiac output response during dynamic exercise.

In the third project, the mechanism of the immediate increase in central venous pressure or right atrial pressure at exercise onset was investigated. Responses of heart transplant patients were compared to normal subjects since these patients provide a model for isolating peripheral changes and their influence on central venous pressure from changes in cardiac function at exercise onset. The immediate increase in central venous pressure with leg movement in both groups of subjects indicated a central shift in blood volume due to muscle contraction. Central venous pressure continued to increase over the next 3 minutes in heart transplant but not in normal subjects due to the slower component of reflex cardiac adjustment in the former group as exercise continued. This study supports a role for the muscle pump in circulatory control at exercise onset but illustrates the importance of

the interaction between cardiac response and circuit function in determining central venous pressure as exercise continues.

In the fourth project I determined whether altering the metabolic signal during exercise alters cardiac response. I studied the heart rate-oxygen consumption relationship and daily energy expenditure in female patients who significantly altered their body mass, and thus their oxygen consumption at a given work rate, following surgical treatment for morbid obesity. They were compared to obese patients before surgery, patients who failed to lose weight following surgery and non-obese control subjects. I hypothesized that: 1) heart rate would follow relative oxygen consumption; 2) peak absolute oxygen consumption would not change as a result of weight loss if activity level was the same; 3) a failure to lose weight following surgery may be due to a reduced normalized energy expenditure. Heart rate increased during graded exercise in proportion to the relative oxygen consumption in all groups which indicates that control of heart rate is unaltered by changes in the metabolic response to work. However, despite a similar normalized daily energy expenditure, peak oxygen consumption decreased in patients who successfully normalized their body mass compared with all other groups. This suggests a reduction in fat-free mass. In addition, reduced energy expenditure was not a factor in failure to lose weight following surgery. The latter two findings are of clinical significance in this population and suggest that large rapid

weight loss reduced aerobic capacity. As well, factors other than energy expenditure must account for failure to lose weight following surgical treatment. This study supports the hypothesis that heart rate is a function of relative effort during dynamic exercise and gives clinical insight into the impact of surgical intervention for obesity on energy expenditure and the metabolic response to exercise.

In the fifth project, I focused on changes in peripheral vascular venous mechanics which must contribute to the matching of cardiac output to the increased metabolic demand during dynamic exercise. I used a canine model where dynamic exercise was simulated by intermittent electrical stimulation of the sciatic nerves. The contribution of thin fibre muscle afferents to changes in venous mechanics and cardiac output was separated from the mechanical effect of muscle contraction by blocking neural feedback by cooling the nerves. Use of an anesthetized animal preparation excluded the influence of central command on the variables studied. I tested the hypothesis that a decrease in venous capacitance and resistance would result more from reflex adjustments than recruitment of volume during muscle contractions and no change or a small decrease in venous compliance results from the mechanical effect of muscle contraction. Therefore the overall effect of the muscle pump on changes in venous mechanics and cardiac output during exercise would be less important compared with reflex effects mediated by thin fibre muscle

afferents which result in sympathetic activation. In nerve intact animals, cardiac output, heart rate and arterial pressure increased in response to exercise. However, these changes were suppressed in nerve blocked animals. The increased gradient for venous return, which resulted from an increase in mean circulatory filling pressure and the tendency for a decrease in right atrial pressure during stimulation, was also reversed in nerve blocked animals. The magnitude of increase in mean circulatory filling pressure was similar in both nerve intact and blocked conditions, however, only the former was statistically significant. Venous compliance and resistance did not change in nerve intact animals, although the latter decreased in all but one animal when the exercise stimulus was repeated under conditions of increased autonomic tone during a head-up tilt manoeuvre. This study suggests that increases in heart rate and cardiac output in this model of simulated mild dynamic exercise occur in response to reflex adjustments mediated by thin fibre muscle afferents. Increased cardiac output is achieved by an increase in the pressure gradient for venous return, through a muscle afferent-mediated increase in pump function and increase in mean circulatory filling pressure. There is likely also a small mechanical effect of muscle contraction on mean circulatory filling pressure. Overall venous compliance and resistance are unaffected by mild simulated dynamic exercise, however, a decrease in resistance to venous return may contribute to

increases in cardiac output under conditions of decreased autonomic reserve.

In summary, these conclusions support an important role for peripheral vascular factors in the control of cardiac output during the integrated response to dynamic exercise. Neural, humoral and mechanical mechanisms contribute to specific individual determinants of venous return. Neural feedback from muscle afferents provides the signal which increases the pressure gradient for venous return and cardiac output. Changes in arterial baroreceptor input and local changes at the vessel wall can have opposite effects on different blood-borne vasoconstrictor peptides involved in blood flow distribution. The mechanical effect of muscle contraction is important for shifting blood centrally at exercise onset, perhaps to allow for a relatively rapid increase in cardiac output, and may influence circuit function during mild dynamic exercise. However, the interaction between cardiac and peripheral circuit function determines the central venous pressure at a given cardiac output. Heart rate remains a function of relative effort despite altered metabolic response to exercise and cardiac output remains matched to the oxygen cost of exercise despite cardiac denervation. This suggests that heart rate and cardiac output are controlled variables during dynamic exercise which increase as sympathetic outflow is increased to the heart and, equally important for cardiac output, to the periphery. The latter results in alterations in

the peripheral vasculature which favour an increase in venous return and cardiac output. These sympathetically mediated reflex adjustments are due in large part to afferent signals from thin fibre muscle afferents although regional vascular responses may be influenced either directly or indirectly by the arterial baroreceptor. The scope of peripheral vascular adjustments may be a major limiting factor to maximal oxygen consumption rather than the pumping capacity of the heart.

CLAIMS TO ORIGINALITY

The following are original contributions towards understanding peripheral vascular vs cardiac factors in the control of cardiac output during exercise.

1) The carotid baroreflex controls plasma neuropeptide Y levels but does not affect plasma endothelin-1 levels in vivo. The former supports and the latter is in contrast to suggestions in the literature based on indirect evidence.

2) Plasma endothelin-1 levels increase in response to systemic hypotension through local mechanisms when vagi are cut but not when they are intact suggesting an inhibitory vagal influence. This indicates that neurohumoral interactions influence these circulating vasoactive peptides involved in blood flow distribution.

3) Cardiac function is not the sole limit to exercise in heart transplant patients which suggests that adjustments in the peripheral circuit which affect venous return contribute to maintaining a normal increase in cardiac output with increasing oxygen consumption in spite of cardiac denervation.

4) The mechanical effect of muscle contraction accounts for the increase in central venous pressure immediately at exercise onset, whereas adaptations in cardiac response determine central venous pressure and prevent a further rise as exercise continues. This suggests that peripheral mechanical factors augment venous return at exercise onset and

this precedes neurally-mediated changes in cardiac function.

5) Heart rate is a function of the relative oxygen consumption in patients who significantly altered their oxygen consumption at a given work rate through large changes in body mass following surgical treatment for morbid obesity. This result supports previous training studies and experiments comparing exercise with large vs small muscle mass and suggests that control of heart rate is unaltered by changes in the metabolic response to work.

6) Unexpectedly, subjects who normalized their body mass one year following surgical treatment for morbid obesity had a significant decrease in peak oxygen consumption perhaps due to a reduced fat free mass, although this was not evident at six months post surgery. This finding was not due to a reduced normalized energy expenditure and neither was the failure to lose weight following surgery. This is of clinical relevance to functional outcome following surgery in this population.

7) The pressure gradient for venous return was increased during dynamic pattern muscle stimulation but not when feedback from thin fibre muscle afferents was blocked, although the upstream pressure for venous return was increased under both blocked and unblocked conditions. This was due to reflex effects in response to muscle afferent stimulation which contribute to changes in cardiac function and a possible small mechanical effect of muscle contraction on the upsteam pressure for venous return. Both mechanisms likely contribute

to increases venous return and cardiac output during dynamic exercise.

8) Venous compliance and resistance to venous return were unaltered during muscle stimulation which simulated mild dynamic exercise. However, resistance to venous return tended to decrease when exercise was stimulated in a more upright posture and autonomic reserve was decreased. This suggests that changes in venous mechanics may vary with the level of sympathetic outflow during dynamic exercise.

9) Cardiac output and heart rate increases in response to simulated dynamic exercise were abolished when thin fibre muscle afferents were blocked. This suggests that these afferents are the major signal matching heart rate and cardiac output responses to increases in metabolic demand during mild dynamic exercise.

10) Taken together, these results suggest that changes in peripheral vascular factors which are a function of neural, humoral and mechanical influences, have a large effect on cardiac output during dynamic exercise. These interact to produce the controlled increase in venous return and cardiac output which are matched to increases in oxygen consumption.

APPENDIX I

SI Unit Equivalents

1 Pascal (Pa) = 0.075 mmHg
= 0.01 cmH2O

APPENDIX II

Abbreviations

α	alpha
µl	microlitres
a-vO2	arterio-venous oxygen content difference
BMI	body mass index
BMR	basal metabolic rate
bt/min	beats per minute
C	celsius
CaO2	arterial concentration of oxygen
CO	cardiac output
cpm	cycles per minute
CS	carotid sinus
Ct	total compliance
CvO2	mixed venous concentration of oxygen
CSP	carotid sinus pressure
CVP	central venous pressure
dl	decilitres
DO2	oxygen diffusing capacity
EDRF	endothelial derived relaxing factor
EDTA	ethylenediaminetetraacetic acid
EE	energy expenditure
et.al.	and co-authors
ET-1	endothelin-1
exEE	exercise energy expenditure
Ff	fraction of flow to fast time constant bed
Fs	fraction of flow to slow time constant bed
Hg	hemoglobin
HR	heart rate
HT	heart transplant
i.e.	that is
irET-1	immunoreactive endothelin-1
i.v.	intravenous
kg	kilogram
l	litres
MCFP	mean circulatory filling pressure

mg	milligrams
min	minutes
ml	millilitres
mmHg	millimetres of mercury
mmol/l	millimole per litre
mol/l	moles per litre
MSNA	muscle sympathetic nerve activity
NPY	neuropeptide Y
Pa	arterial pressure
PAO2	alveolar partial pressure of oxygen
PaO2	arterial partial pressure of oxygen
PcO2	capillary partial pressure of oxygen
Pcs	carotid sinus pressure
pg	picograms
PmitoO2	mitochondrial partial pressure of oxygen
Pms	mean systemic pressure
PO2	partial pressure of oxygen
Ppa	pulmonary artery pressure
Pra	right atrial pressure
PTO2	tissue partial pressure of oxygen
PvO2	venous partial pressure of oxygen
Pw	pulmonary capillary wedge pressure
$\dot{Q}$	flow
RVO2	resting oxygen consumption
rpm	revolutions per minute
Rv	resistance to venous return
SAP	systemic arterial pressure
S.D.	standard deviation
S.E.	standard error of the mean
SV	stroke volume
TDEE	total daily energy expenditure
Tf	fast time constant
Ts	slow time constant
v	stressed volume
Vo	unstressed volume
$\dot{V}O2$	oxygen consumption
$\dot{V}O2max$	maximal oxygen consumption
V.R.	venous return