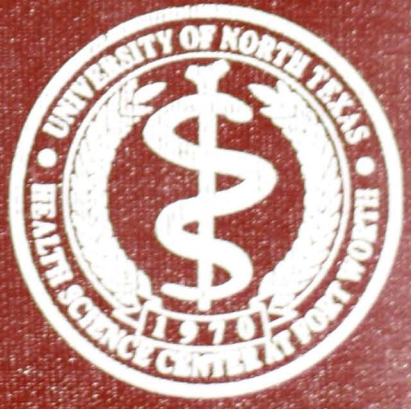
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No data exist in the literature describing the myocardial oxygen supply/demand relationship of the right ventricle in a conscious, unanesthetized animal. A novel technique developed in our laboratory enables us to collect right ventricular (RV) venous blood samples from conscious dogs to determine RV myocardial oxygen consumption (MVO_2). RV oxygen supply/demand balance was examined in conscious dogs, chronically instrumented to measure right coronary blood flow (RCBF), segmental shortening (%SS) and RV pressure (RVP) during increases and decreases in RV myocardial oxygen demand. Right ventricular MVO_2 and O_2 extraction (O_2E) were determined; RCBF, RVP, dP/dt, and %SS were recorded concomitantly. Acute increases in RV MVO_2 were accomplished by atrial pacing (200 beats/min), increasing RV afterload by 65%, infusion of isoproterenol (0.1 μg/kg/min, *i.v.*), and by conducting a submaximal exercise routine (70-75% of maximum VO_2). An acute decrease in RV MVO_2 was created by propranolol administration (1 mg bolus, *i.c.*). During acute increases in RV MVO_2, the extraction reserve is utilized primarily; flow is not affected in the absence of direct vasodilatory effects of the intervention. A decrease in RV oxygen demand is associated with a further increase in the RV extraction reserve. Since RV O_2E increases linearly with increases in RV MVO_2, these data show that changes in

RV venous O_2 tension can occur with little or no change in RCBF. LC resistance is very sensitive to alterations in LC venous pO_2; therefore, there appear to be significant differences between the left and right ventricles concerning the matching of oxygen supply with myocardial oxygen demand.

MECHANISMS OF RIGHT VENTRICULAR OXYGEN

SUPPLY/DEMAND BALANCE IN THE

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MECHANISMS OF RIGHT VENTRICULAR OXYGEN SUPPLY/DEMAND BALANCE IN THE CONSCIOUS DOG

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By

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INTRODUCTION

In concluding the chapter comparing right and left ventricular coronary perfusion in his book entitled *The Coronary Circulation in Health and Disease*, Melvin L. Marcus wrote, "...many differences exist between the regulation of perfusion to the right and left ventricles. All too often, these differences are ignored by investigators and clinicians. Discrepancies in the regulation of perfusion to the right and left ventricles deserve much more attention than they have been given in the past" (37). Although Marcus' caveat was published in 1983, many conclusions are still derived from contemporary investigations without consideration of ventricular differences.

Right and Left Ventricular Differences

Extrapolation of the conclusions derived from left ventricular (LV) data to include right ventricular (RV) responses is not always accurate. This is not surprising, considering that the left and right ventricles differ in many respects, including basal metabolism, oxygen demand, and myocardial perfusion. RV systolic pressure is considerably lower, and the mass of the ventricular free wall is much smaller. Since the RV has a lower energy demand than the LV, the RV

does not require as large of an energy reserve; the cytosolic phosphorylation potential {P.P. = [ATP]/[ADP][P_i]} is reduced compared to that of the LV (29). Vascular smooth muscle tone is also substantially different among the ventricles, as basal right coronary (RC) resistance is much higher than the left coronary (LC) vessels (56).

The dynamic control of RC vascular tone can be partially described by the autoregulatory response. The LV has extremely potent autoregulation over a wide range of coronary perfusion pressures (CPP) (63), while the autoregulation of RC flow is less effective (4, 63, 64). The mechanisms responsible for the discrepant pressure/flow relationships have not been determined. In addition to the autoregulatory characteristics, another essential component required to describe coronary vascular control is local matching of oxygen supply with oxygen demand.

Oxygen Supply/Demand Balance

The oxygen demand of the working myocardium is affected by the afterload and systolic developed pressures, wall stress, heart rate, inotropic state, coronary perfusion pressure, and even oxidative substrate selection (3, 19, 23, 32, 52, 59). A sustained increase in oxygen demand will necessitate a concomitant increase in oxygen supply to prevent myocardial necrosis. The oxygen demand of the myocardium is reflected by the myocardial oxygen

consumption (MVO_2) as long as O_2 supply is not limited (42). The oxygen consumption is determined by coronary blood flow (CBF) and percent oxygen extraction {O_2E, (O_2E = [(O_2 $Content_{arterial}$ – O_2 $Content_{venous}$) / O_2 $Content_{arterial}$] * 100)}. The relationship between the variables of MVO_2, CBF, and O_2E can be described as the oxygen supply/demand balance of the myocardium.

MVO_2 and Coronary Blood Flow

The rate of oxygen consumption by the RV is significantly lower than that of the LV, as the right heart performs less internal and external work (26, 32, 55). Investigations of MVO_2 in anesthetized dogs have reported basal RV values from 3.5 to 5.3 ml O_2/min/100g (17, 50-53, 55, 63). In similar preparations, basal LV MVO_2 was found to range from 7.8 to 9.6 ml O_2/min/100g (32, 51, 57). Similarly, basal RV coronary blood flow (CBF) values in the anesthetized dog range from 0.46 to 0.66 ml/min/g (6, 19, 32, 39, 55), whereas LV values are 0.75 to 1.1 ml/min/g (7, 32, 55, 57). Conscious dogs tend to have slightly higher basal CBF, with values as high as 0.78 in the right (40, 41), and as high as 1.17 in the left (2, 12, 40). The lower CBF in the anesthetized animals is probably due to the cardiodepressive effects of anesthesia (see below). In general, right CBF is 60-65% that of the left, in accordance with the significantly lower MVO_2. However, the ratio of CBF/MVO_2 is higher in RV than in LV. This difference is associated with lower RV O_2E.

Coronary Flow Reserve

The right ventricular vessels, which display a higher basal coronary resistance, exhibit similar or even higher maximal dilatory capacity than the left ventricular vessels (45). Since basal coronary flow is much lower and maximal vasodilatory capacity is similar, it follows that the RV has a much larger flow reserve (53, 61). Studies have demonstrated that this coronary vasodilatory reserve of the right ventricle is not maximally utilized even during severe exercise, coronary hypoperfusion, or in the presence of right ventricular hypertrophy or pulmonary artery constriction (6, 21, 39, 41, 44).

Oxygen Extraction Reserve

PO_2 and O_2 content of RV venous blood samples are significantly higher than that of the left ventricle, a consistent finding in the literature (3, 26, 32, 36, 39). The result is a low RV baseline O_2E of about 43%, upon which the RV can rely when MVO_2 is increased. In contrast, the left ventricle, with basal O_2E values as high as 79%, has a limited ability to increase extraction (32, 53, 55, 58). The extraction reserve of the left ventricle has been shown to be effectively depleted by relatively small reductions in myocardial blood flow (46) or increases in oxygen demand (32, 36, 58), and further reductions in the oxygen supply/demand ratio necessitate an increase in CBF.

Anesthetized vs. Conscious Preparations

Although anesthetized animals provide invaluable results, there is concern regarding effects of anesthesia and open chest procedures. The combined effects of general anesthesia and open-chest perturbations have been shown to alter coronary autoregulation, basal vascular tone, myocardial flow distribution, coronary hemodynamic measurements, myocardial function, cardiovascular reflex pathways, infarct size, and even the Frank-Starling mechanism (5, 7-9, 30, 31, 33, 54). Vatner wrote in 1978, "While general anesthesia affects almost every aspect of the circulatory system, the importance of general anesthesia on the circulation tends to be underestimated by considering only its direct effects.... Major differences, often directionally opposite in responses of conscious and anesthetized animals, were found for reflex control of the circulation, effects of hemorrhage, and alterations in preload and afterload" (60).

RC Venous Blood Sample Collection

RC venous blood samples, which are necessary for determining mechanisms responsible for maintaining RV oxygen supply/demand balance, are difficult to obtain. The anatomy of the RV does not provide a common area of drainage, such as the coronary sinus for the LV, as the RV superficial veins drain directly into the right atrium. This fact necessitates collection of RV blood samples from along the main body of superficial veins. The numerous superficial veins draining the area perfused by the right coronary artery (RCA) are small and

fragile. Even in the acute, open-chest preparation, it is difficult to maintain the patency of the RC vein and/or the sampling catheter. Most investigations collect RC venous blood samples by allowing the catheter to flow continuously (3, 32, 39, 63). This is not possible in chronic investigations, and obtaining RC venous blood samples is even more challenging. Since the veins cannot drain freely, and since anchoring the catheter at the point of insertion will occlude the vein, the veins are subject to concomitant coagulation. Therefore, no method has been available for the collection of RC venous blood samples in a conscious animal.

As a result, only LV myocardial supply/demand balance has been studied in a conscious animal. Ventricular differences limit the extrapolation of the conclusions derived from this research to include the right ventricle. Thus, investigations aimed at RV oxygen supply/demand balance in a conscious animal during alterations in RV MVO_2 are necessary.

Interventions that increase MVO_2

Acute increases in MVO_2 can be induced by catecholamine infusion (6, 17, 23, 27, 32, 48, 50-53), atrial pacing (20, 32, 38, 43, 47, 49, 50, 62), pulmonary artery constriction (19, 20, 32), and exercise (2, 13, 15, 16, 24, 28, 43). These interventions increase the MVO_2 of both ventricles, and CBF and O_2E will be affected.

Isoproterenol

Intravenous infusion of isoproterenol at a rate of 0.1 μg/kg/min in the anesthetized dog will increase RV MVO_2 by an average of 162%, while RCBF increases 128% and O_2E increases 19% (32, 51, 52). In this condition, it appears that the RV myocardium increases both O_2 extraction and flow to meet the increase in O_2 demand (32, 51, 53). The LV, however, with basal O_2E near 60%, appears to be unable to increase extraction substantially during isoproterenol infusion, relying completely on an increase in CBF. This finding is supported by several investigations in anesthetized and conscious subjects. These studies found no change (10, 32, 48) or a decrease (22, 50) in LV O_2E during isoproterenol infusion. Therefore, LC vascular smooth muscle may be more responsive to β-receptor mediated vasodilation. In addition, there is evidence that the LC vasculature is more responsive to metabolic vasodilation than the RV (18). There appears to be major differences in the response to isoproterenol infusion between the right and left ventricles.

Atrial Pacing

Atrial pacing is another method utilized to cause specific rate-induced increases in RV MVO_2, and a few studies have demonstrated increases in RV MVO_2 in the anesthetized dog. Two such investigations paced the heart at a rate of 175 and 225 beats/min, resulting in a 31 and 100% increases, respectively, in RV MVO_2 (32, 62). RCBF data collected during pacing are less precise, with

reports of no change to a 130% increase during pacing at a rate of 200 beats/min (32, 43, 47, 62). Adding to the confusion, O_2E values have been reported ranging from a 31% increase while pacing at 175 beats/min to a 25% decrease during pacing at 225 beats/min (32, 62). RV MVO_2 and O_2E have not been determined during pacing in a conscious animal.

Increased Pulmonary Artery Pressure

Constriction of the pulmonary artery results in an acute increase in RV systolic pressure and MVO_2 (19, 20, 32). The RV appears to rely on its oxygen extraction reserve during this condition (32). RCBF may also be increased (20, 32), although this finding is not definitive (19). The relative contributions of RV oxygen extraction and flow reserves during pulmonary artery constriction have not been determined in a conscious animal.

Exercise

Exercise is known to increase MVO_2 due to an increase in heart rate, contractility, stroke volume, and circulating catecholamines, leading to large increases in CBF and O_2E (1, 11, 13, 14, 16, 24). However, Duncker et. al recently demonstrated in the exercising pig that the large increase in LV MVO_2 due to exercise was met entirely by an increase in CBF, as the O_2E and PvO_2 were unchanged (12). Although RV studies have found highly variable increases in RCBF depending on the level of exercise (2, 34, 35, 43, 45), no method of

right coronary venous blood sampling has been available for the determination of RV MVO_2 and O_2E during exercise.

Interventions the decrease MVO_2

An acute decrease in myocardial oxygen consumption can be induced by negative inotropic agents, such as propranolol (17, 25). One investigation reported that intravenous administration of 3 mg/kg propranolol in the anesthetized dog decreased the heart rate and RV dP/dt (17). RV MVO_2 would have decreased accordingly, but was not reported in this investigation. During this decrease in demand, RCBF decreased 13%. The effects on coronary vascular resistance were unclear, however, as mean arterial blood pressure was reduced more than 20%. No investigation has examined the mechanisms of RV oxygen supply/demand balance during propranolol administration in the right ventricle of conscious animals.

Summary

All of these perturbations have resulted in new data defining the hemodynamic and metabolic responses of the ventricles to increases or decreases in oxygen demand and to decreases in oxygen supply. LV oxygen extraction is high at rest (26, 32, 36). Thus, the LV oxygen extraction reserve is small, and it contributes minimally to LV oxygen consumption during increases in LV oxygen demand. On the other hand, the RV has a large extraction reserve at

rest (3, 26, 32, 36, 39), and this reserve might be mobilized to contribute significantly to RV oxygen consumption during increases in RV oxygen demand. The RV also has a large RC flow reserve (53, 61), and several studies have observed RC flow increases during increases in RV MVO_2 (21, 32, 52). Interactions between RV flow and extraction reserves during alterations in RV oxygen demand have not been described in the conscious animal. These experiments were, therefore, designed to generate new understanding of RV oxygen/supply demand balance during interventions that alter RV oxygen demand. The protocols were developed to examine RV MVO_2, RCBF, and RV O_2E during atrial pacing, isoproterenol infusion, acute pulmonary artery pressure increases, propranolol administration, and a graded exercise regimen.

Specific Aims

The first aim of this investigation was to determine the relative contributions of oxygen extraction and coronary flow reserves during acute increases in RV oxygen demand.

Increases in oxygen demand at rest were created by atrial pacing (200 beats/min), intravenous infusion of isoproterenol (0.1 μg/kg/min), and inflation of a pulmonary artery balloon catheter (PAC, systolic RV pressure increased from 20±1 to 33±1 mmHg). We hypothesized that RV oxygen extraction would be utilized in preference to the coronary flow reserve. We found that, during pacing

and PAC, the oxygen extraction was increased with no significant change in CBF. The large increase in RV MVO_2 associated with isoproterenol infusion resulted in increases in RCBF and RV O_2E. RC venous pO_2 was substantially decreased during the increases in RV MVO_2 associated with pacing, PAC, and isoproterenol infusion. Therefore, we concluded that the right ventricle has a large oxygen extraction reserve under resting conditions, and will utilize this source of oxygen supply preferentially to the large flow reserve in the absence of direct vasodilatory effects of the intervention. We also concluded that right coronary resistance is not affected by alterations in venous pO_2 over a large range when an oxygen extraction reserve is available, quite different than the left coronary circulation. Major differences appear to exist between the ventricles concerning the local control of matching oxygen supply with oxygen demand.

The second aim of this investigation was to determine the relative alterations of oxygen extraction and coronary flow reserves during acute decreases in RV oxygen demand caused by intracoronary administration of propranolol. We hypothesized that when RV MVO_2 is decreased, coronary blood flow would not significantly change while the RV extraction reserve would be enhanced. We found that when RV MVO_2 decreased 15%, RCBF was unaffected and RV O_2E decreased 15%; RC venous pO_2 was significantly higher. We concluded that acute decreases in RV MVO_2 result in equivalent increases in RV O_2E reserve without affecting RCBF.

Significance

Our findings have resulted in the first data ever collected describing RV oxygen supply/demand balance in a conscious animal. The information has resulted in a greater understanding of RV vascular responses to alterations in oxygen demand. The results have led to the conclusion that significant differences exist between right and left ventricular mechanisms that match coronary blood flow to oxygen demand. Several pathologic conditions exist that involve the right ventricle, such as pulmonary hypertension, RV hypertrophy, and right coronary artery stenosis. With the basic understanding of the mechanisms of RV oxygen supply/demand balance in normal subjects, pathologic alterations of these mechanisms may be determined. Therapeutic interventions may then be aimed at correction of the mechanism responsible for the oxygen supply/demand mismatch.

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CHAPTER II

MECHANISMS OF RIGHT VENTRICULAR OXYGEN SUPPLY/DEMAND BALANCE IN THE CONSCIOUS DOG

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Abstract

No data exist in the literature describing the myocardial oxygen supply/demand relationship of the right ventricle in a conscious, unanesthetized animal. A novel technique developed in our laboratory enables us to collect right ventricular (RV) venous blood samples from conscious dogs to determine RV myocardial oxygen consumption (MVO_2). RV oxygen supply/demand balance was examined in conscious dogs, chronically instrumented to measure right coronary blood flow (RCBF), segmental shortening (%SS) and RV pressure (RVP) during increases and decreases in RV myocardial oxygen demand. Right ventricular MVO_2 and O_2 extraction (O_2E) were determined; RCBF, RVP, dP/dt, and %SS were recorded concomitantly. During acute increases in RV MVO_2, the extraction reserve is utilized primarily; flow is not affected. A decrease in RV oxygen demand is associated with a further increase in the RV extraction reserve. These data show that changes in RV venous O_2 tension can occur with little or no change in RCBF. There appear to be significant differences between the left and right ventricles concerning the matching of oxygen supply with myocardial oxygen demand.

Key words: oxygen supply/demand balance, right ventricle, pacing, isoproterenol, propranolol, pulmonary arterial pressure

Introduction

The right ventricle (RV) generates a much lower systolic pressure than the left ventricle (LV), and consequently the RV has a lower oxygen demand. Few investigations have focused on right coronary (RC) vascular control and oxygen supply/demand balance, and, to date, no investigations have reported on these issues in a conscious animal. Recently our laboratory developed a procedure to obtain RC venous samples from conscious dogs (2). Measurements of O_2 content in these samples and in arterial blood enable RV oxygen demand to be determined. This procedure was used to investigate RV oxygen balance in conscious dogs.

Myocardial oxygen consumption (MVO_2), coronary blood flow (CBF), and percent oxygen extraction are key variables used to evaluate O_2 supply/demand relationships. MVO_2 reflects the oxygen demand of the myocardium when oxygen is not limited, and CBF and O_2E reflect the coronary vascular response to this oxygen demand. This study was designed to define contributions to changes in MVO_2 of RC flow and O_2E reserves, which are available to affect myocardial oxygen supply when RV oxygen demand is altered.

Numerous investigations have documented the effects of altered RV and LV oxygen demand in anesthetized, open chest animal preparations. Substantial

data exist in the literature regarding the effects of atrial pacing (10, 16, 30), afterload increases (9, 10, 16), catecholamine infusion (12, 16, 24), and propranolol administration (8, 13). All of these perturbations have resulted in data describing the hemodynamic and metabolic responses of the ventricles to increases or decreases in oxygen demand. LV oxygen extraction is high at rest (14, 16, 17). Thus, the LV oxygen extraction reserve is small, and it contributes minimally to increases in LV oxygen consumption during increases in LV oxygen demand. On the other hand, the RV has a large extraction reserve at rest (1, 14, 16, 17, 19), and this reserve might be mobilized to contribute significantly to RV oxygen consumption during increases in RV oxygen demand. The RV also has a large RC flow reserve (25, 29), and several studies have observed RC flow increases during increases in RV MVO_2 (11, 16, 24). Interactions between RV flow and extraction reserves during alterations in RV oxygen demand have not been described in the conscious animal.

In this investigation, we observed that the RV extraction reserve was preferentially utilized to increase oxygen supply during increases in RV oxygen demand. During decreases in oxygen demand, the RV oxygen extraction reserve was enhanced. In addition, the local control of coronary blood flow appears to differ between the ventricles. Subtle decreases in venous oxygen tension are associated with increases in LV CBF, whereas RC venous pO_2 may vary widely without changes in RCBF.

Materials and methods

Animal instrumentation

This investigation was approved by the Institutional Animal Care and Use Committee and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1996). A total of thirteen adult mongrel dogs of either sex, weighing 24 to 32 kg were studied. Fifteen were attempted; two animals were terminated prematurely due to internal bleeding. Thirty minutes after preanesthesia treatment with PromAce (0.03 mg/kg, *i.m.*), anesthesia was induced by thiopental sodium (5 mg/kg, *i.v.*). Following endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with isoflurane gas (1-3%) with equal offset of oxygen (1 L). Under sterile conditions, a thoracotomy was performed in the fourth right intercostal space, and the dog was instrumented as illustrated in Fig 1. A Tygon catheter (0.04 in i.d., 0.07 in o.d.) was inserted into the aorta through the right internal mammary artery to measure aortic pressure. A similar Tygon catheter was implanted in the right atrium to infuse isoproterenol. A Konigsberg model P6.5 pressure transducer was inserted through a stab wound in the RV infundibulum and secured with a purse string suture. A nonbranching section of the right coronary artery (RCA) was dissected free for 1 - 2 cm to affix a Transonic® flow transducer. A Micro-Renathane catheter (0.014 in i.d., 0.033 in o.d.) was inserted into the RCA distal to the occluder through a small side branch

to the right atrium and advanced into a proximal portion of the RCA. A coronary venous catheter prepared from Micro-Renathane tubing (type MRE-025, 0.012 in i.d., 0.025 in o.d.) was inserted into a superficial vein draining the RV myocardium, as described previously (2). To measure RV segment shortening, a pair of piezoelectric crystals was inserted into the middle wall of the RV. These crystals were positioned 1 cm apart and perpendicular to the main RCA. A pacing wire was sutured to the right atrial appendage. A Baxter® 8F Fogarty arterial embolectomy catheter (80 cm length) was inserted through a puncture wound in the main pulmonary artery and secured with a purse-string suture. The balloon tip, once inflated, had a diameter of 1.5 cm, sufficient to increase the afterload of the RV.

At the conclusion of instrumentation, catheters and wires were brought out of the thorax through the 3rd and 5th right intercostal spaces, tunneled under the skin, and exteriorized between the shoulders through individual puncture wounds. The chest was closed, and the pneumothorax evacuated through a chest tube. Antibiotic (Clavamox®, 6.25 mg/lb, b.i.d., p.o.) and aspirin (162 to 300 mg, p.o.) were given for 10 days after surgery. The RC venous catheter was attached to an Access Technologies "C" Series® Balloon Pump (60 ml volume elastomeric pump, 2.0 ml/hour flow rate) for continuous infusion of heparinized saline (10 units/ml) for the duration of experimentation. The RC catheter was

flushed with heparinized saline (10 units/ml) and filled with heparin (5000 units/ml) daily. Other catheters were treated similarly at three-day intervals.

Data collection

After recovery from surgical procedures, measurements were obtained with the animal standing quietly in a sling. The interventions described below were performed in random order over a two-week period. Interventions conducted on the same day were initiated at least 30 minutes apart to allow recovery to baseline values. The half-life of propranolol is from 3-6 hours; this treatment was the terminal intervention for a given day.

RC flow was measured with a Transonic® T106 series flowmeter. A pressure transducer (Narco Telecare® model LDI-5) was positioned at mid-heart level and connected to the mammary artery catheter to measure AoP. Ultrasonic signals from length dimension crystals were processed by a Triton Technology model 120 sonomicrometer and monitored with a Tektronics model 2215A oscilloscope. Segment lengths at the beginning of the positive deflection of the dP/dt record were considered end diastolic, and those measured 20 ms before the peak negative deflection were considered end systolic, as previously described (28). RC flow, right ventricular pressure (RVP), dP/dt, systemic arterial blood pressure (AoP), and RV segment length were recorded on a multichannel Grass® model 7D Polygraph when RC flow and arterial blood pressure had

stabilized for at least 30 s at each intervention. Data were then extracted by hand from the recordings. Segment shortening was calculated as a percent by the formula: {%SS = (end diastolic length – end systolic length) / (end diastolic length)*100}. Right coronary resistance was calculated by dividing mean AoP by RCBF. Oxygen extraction was determined by the formula: {O_2E = [(O_2 $Content_{arterial}$ - O_2 $Content_{venous}$) / O_2 $Content_{arterial}$] * 100}. During analysis of the isoproterenol data, we wanted to determine the relative contributions of RCBF and A-V O_2 content difference to the observed increase in RV oxygen consumption. It was determined that the percent contribution of each variable was directly proportional to the percent increases from respective pre-treatment values.

Plasma Sample Collection and Analyses

Arterial and venous blood samples were collected to determine oxygen content, pO_2, and glucose and lactate concentrations. Arterial samples were collected from the mammary arterial catheter, and venous samples were collected from the implanted RC venous catheter. All samples were collected anaerobically and chilled on ice until analyses. Oxygen content and pO_2 were determined by an Instrumentation Laboratory Co-Oximeter and a Synthesis 30 Blood Gas Analyzer, respectively. MVO_2 was determined by multiplying the arteriovenous difference of oxygen content by the coronary blood flow, normalized per gram tissue mass. Blood glucose and lactate concentrations were

determined using a Yellow Springs Instruments® Model 2300 STAT Plus Glucose and L-Lactate Analyzer. Glucose and lactate uptakes were calculated by multiplying the arteriovenous substrate difference by the normalized coronary blood flow.

Experimental Interventions

Pacing (n=6). The canine subject was placed in a sling as described above. After the collection of baseline arterial and coronary venous blood samples, atrial pacing was initiated at a rate of 200 beats/min. The rate and magnitude of pacing potentials were controlled with a Grass Instruments® S44 stimulator. Arterial and venous samples were collected after stabilization of AoP and RCBF at 3 min.

Isoproterenol infusion (n=6). The isoproterenol (Isuprel®) was diluted with sterile saline into a 10 ml syringe to infuse over a 10 min period (0.1 μg/kg/min). This infusion was initiated into the right atrial catheter after the collection of baseline measurements with the animal resting quietly in a sling. Arterial and venous samples were collected and recordings made after stabilization of AoP and RCBF variables during the last 3 minutes of the infusion period.

Propranolol infusion (n=6). Baseline blood samples and hemodynamic recordings were obtained with the dog resting quietly in a sling. Propranolol

hydrochloride (Inderal®, 1 mg bolus) was then injected into the coronary arterial catheter to create a non-selective β-receptor blockade of the RV myocardium leading to a decrease in oxygen demand. After a stabilization period of at least 15 minutes, data were again recorded as blood samples were withdrawn.

PA balloon catheter inflation (n=8). Blood samples were taken and recordings made with the dog resting quietly in a sling for baseline measurements. Inflation of the Fogarty arterial embolectomy catheter then increased peak systolic RVP from 20±1 to 33±1 mmHg ($p<0.05$). After stabilization of hemodynamic variables, blood samples were again collected before release of the balloon inflation.

Measurement of RCA Perfusion Territory

After termination of the experiments, the animal was euthanized with sodium pentobarbital (30 mg/kg, i.v.) followed by potassium chloride. After opening the chest, approximately 15 ml of 2.5% Evan's Blue dye was injected with a syringe into the RCA catheter to delineate the perfused territory. This territory was then carefully excised and weighed to normalize the coronary flow per gram of tissue mass.

Statistical Analyses

All values are expressed as means ± SEM. A paired t-test was performed to detect possible differences between baseline and treatment values. Analyses were performed and interpreted according to Zar (32). Statistical significance was assumed at $p<0.05$.

Results

Table 1 presents hemodynamic and metabolic data of the four treatments affecting the oxygen demand of the myocardium. Mean aortic blood pressure was not significantly affected by any of the treatments. Heart rate was increased by isoproterenol infusion, but was not altered by PAC inflation or propranolol bolus. Peak systolic RVP was slightly decreased by pacing and propranolol. This variable was significantly increased by PAC inflation and isoproterenol infusion. Regional contractile function was diminished by all treatments with the exception of isoproterenol infusion, which resulted in enhanced segmental shortening.

The oxygen tension of the RC venous blood was also significantly affected by all treatments that altered oxygen demand. The three interventions that increased demand resulted in concomitant decreases in venous pO_2; these decreases were associated with significant increases in O_2E. Decreased oxygen demand during propranolol administration resulted in an increased as O_2E decreased.

Glucose uptake was unaffected by pacing, but was higher during increases in demand associated with PA balloon inflation and isoproterenol infusion. Propranolol administration did not affect GU. Lactate uptake was

unaffected by pacing, propranolol, or PA balloon inflation; LU was increased during isoproterenol infusion.

Figure 2 provides information describing the oxygen supply/demand balance of the RV during alterations in oxygen demand. With the A-V O_2 content difference on the x-axis and the normalized RC blood flow on the y-axis, the area within the rectangle represents RV MVO_2. Analysis of this data allowed us to determine the contributions of mechanisms responsible for increasing RV oxygen supply to meet demand. As MVO_2 increased 22 and 25% during pacing and PA balloon inflation, respectively, RCBF was not affected as the A-V O_2 difference significantly increased. Increases in RV O_2E were exclusively responsible for meeting the increases in oxygen demand. Isoproterenol infusion created a 77% increase in MVO_2, and resulted in significant increases in RCBF and the A-V O_2 content difference. RCBF provided 83% of the MVO_2 increase, and the increase in O_2E supplied 17%. Propranolol administration created a 15% decrease in MVO_2. RCBF was not significantly affected, while the A-V O_2 difference was enhanced.

The relationship between the variables of RCBF, O_2E, and RV MVO_2 is plotted in Fig 3 for the various interventions affecting oxygen demand. The regression line is indicated (dashed line), and describes the relationships between oxygen supply variables and oxygen demand. Over a large range of

MVO_2, CBF does not increase significantly. Conversely, RVO_2E increases linearly over the same range. During this moderate increase in RV MVO_2, the RV relies exclusively upon increases in oxygen extraction. Differences are observed during isoproterenol infusion (condition 5). Since RCBF was increased by isoproterenol, RV O_2E contributed to a lesser extent to the increase in oxygen supply.

RC resistance was plotted against the coronary venous pO_2 in Fig 4. The interventions aimed at increasing RV MVO_2 resulted in large decreases in venous pO_2. The resistance of the RC circulation (mean AoP/RCBF) was not substantially reduced during these conditions. The large increase in oxygen demand associated with isoproterenol infusion (condition 5) lowered RC resistance and venous pO_2.

Discussion

This report describes to our knowledge the first investigation of RV oxygen supply/demand balance in a conscious, unanesthetized animal. Although substantial data exist concerning LV oxygen handling in the conscious dog, the anatomy of the RV circulation has provided a significant challenge for the collection of coronary venous blood samples. This difficulty is primarily due to the small, fragile superficial veins draining the RV myocardium and the lack of a common drainage area, such as the coronary sinus. Although data have been collected describing RV O_2 extraction and consumption under a variety of conditions in anesthetized, open-chest dogs (15, 31), concerns have been raised regarding the effects of anesthesia and the open chest on the RV, including autoregulation (3, 6) and the distribution of blood flow (26). These issues are more important in light of reports describing differences in autoregulation and O_2 extraction between the right and left ventricles (6, 15). Experiments using a conscious, unanesthetized animal preparation are necessary.

Using our newly developed capability of collecting RC venous blood samples (2), we found that: 1) The RV has a large oxygen extraction reserve under resting conditions, and will utilize this source of oxygen supply preferentially to the large flow reserve. 2) Similarly, decreases in demand do not alter RC resistance, and will increase the extraction reserve available. 3) RC resistance is not affected by alterations in venous pO_2 over a large range, quite

different than the left coronary (LC) circulation. Major differences appear to exist between the ventricles concerning the local control of matching oxygen supply with oxygen demand.

Sample validation

In order for our conclusions to be valid, the RC venous blood samples must contain blood draining the RV myocardium supplied by the RCA. Two possible sources of contamination are the right atrium and blood originating from non-RCA nutritive vessels. To investigate whether there was contamination from right atrial blood, radioactive microspheres were infused simultaneously into the superior and inferior vena cavae. During this time, blood samples were collected from the right atrium and the RC vein and later analyzed for radioactivity. Since the microspheres were trapped within the pulmonary circulation, any radioactivity within the coronary venous samples would have come from right atrial contamination. Mean radioactivity counts (counts/5 min) emitted by the blood samples were 2636±702 for right atrial blood and 2±1for RC venous blood samples. Data from this study demonstrate that there was no right atrial blood withdrawn into the venous samples using our technique.

It is also possible, given the vascular anatomy of the right heart, that blood from the LV circulation may have contributed significantly to RC venous samples. This possibility of venous contamination was explored in an earlier study by

Murakami et al. (19). This group infused Evan's blue dye systemically, while perfusing the RCA from an unlabeled blood supply. This group reported that the LC circulation contributed only a 1.2±1.0 % contamination of RC venous blood samples during RCA normoperfusion (right coronary perfusion pressure = 80 mmHg). This contamination would not have been of detectable significance.

Isoproterenol

Isoproterenol is a synthetic catecholamine that stimulates both β_1- and β_2-adrenergic receptors. This drug increases inotropy and chronotropy of the heart leading to increased oxygen demand, along with arterial dilation. In addition to its clinical usage, many studies have utilized this drug to examine effects of catecholamine stimulation on the RV of anesthetized, open chest dogs (8, 16, 21, 23-25). Saito et al. found that isoproterenol infusion in anesthetized, open chest dogs resulted in a significant increase in LV CBF with a small but significant decrease in the A-V O_2 content difference (22). A later study by the same group in the RV found that infusion of this drug increased RV CBF and the A-V O_2 content difference (23). Therefore, responses to isoproterenol of the coronary circulations appear to differ in RV and LV. A similar isoproterenol infusion was utilized in this investigation. We also found increases in RV CBF and O_2E in response to the increase in MVO_2 associated with this treatment in the conscious dog. Our results describe the response of the RV to isoproterenol administration in the conscious dog.

Propranolol

Propranolol is a nonselective β-adrenergic blocker that inhibits the chronotropic, inotropic, and vasodilator responses to β-adrenergic stimulation and results in a decreased oxygen demand of the myocardium. It is used clinically to reduce the risk of sudden death in patients with acute myocardial infarction, and has been utilized experimentally for β adrenergic blockade (8, 13, 24). In this investigation, propranolol was used to lower the oxygen demand of the RV, and resulted in a 16% decrease in RV MVO_2. Saito et al. administered propranolol in an anesthetized dog and found that LV CBF decreased 30% and the A-V O_2 content difference was not affected (22). In this investigation of the conscious RV, however, we observed a significant decrease in the A-V O_2 content difference with no effect on RCBF. This intervention again demonstrates significant differences in the local control of coronary blood flow between the left and right ventricles.

Pulmonary arterial pressure increases

The impact of pulmonary arterial pressure on RV oxygen handling is especially interesting, with several clinical implications. Numerous investigations have utilized pulmonary artery constriction or banding to create acute or chronic increases in RV afterload (5, 7, 9-11, 16, 18, 20, 25). Saito et al. observed a large increase in LV flow with a significant decrease in A-V O_2 content difference with aortic constriction (22). Other studies in the anesthetized, open chest

preparation have found an increase in RV flow and O_2 extraction with pulmonary artery constriction (16, 23). In our conscious dog, the RV relies completely on increases in extraction to meet increases in demand associated with increases in pulmonary arterial pressure. The reason for this discrepancy is unclear; however, basal MVO_2 measured in this investigation in the conscious dog RV is similar to the MVO_2 induced by pulmonary artery constriction in these two studies. The lower values for basal MVO_2 in the earlier studies can be attributed to the cardiodepressive effects of the anesthesia. In addition, coronary hemodynamics are affected by both anesthesia and the open chest preparation (3, 4, 26).

Right Coronary Vascular Control

The data collected in this investigation provide insight into the local control of the RC circulation. Venous pO_2 is an indicator of interstitial oxygen tension (27). Tissue oxygen tension is a proposed mediator of the local control of the coronary circulation, and in the LV, small reductions in coronary sinus pO_2 result in immediate decreases in LC resistance (27). With the exception of isoproterenol, decreases in RV venous pO_2 did not result in a substantial decrease in RC resistance. Our results support the conclusion that important differences exist between the right and left ventricles regarding the local matching of oxygen supply with demand, at least over the ranges of RV MVO_2 examined in this investigation.

Future studies

To completely describe the oxygen supply/demand balance of the RV myocardium, further investigations will be necessary. Since the LV operates at a much higher basal MVO_2 than does the right, it is difficult to compare the left and right coronary circulations. The relationship between the variables of CBF, venous pO_2, and MVO_2 may be similar in the two ventricles for a given range of MVO_2 values. To determine this possibility, further increases in RV MVO_2 will be required. This could be accomplished by an intervention such as dynamic exercise. The right and left ventricular myocardium may have similar relationships between these variables, but at different operating ranges. However, it is possible that the oxygen supply/demand balance of the RC circulation is fundamentally different from that of the left.

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Table 1. Hemodynamic and metabolic data during treatments.

	AoP (mmHg)	HR (beats/min)	RVP_{max} (mmHg)	SS (%)	PvO_2 (mmHg)	O_2 Extr (%)	GU (μmol/min/g)	LU (μmol/min/g)
Pacing (n=6)	98±4 (101±4)	200* (104±3)	17±1* (20±2)	7.0±0.7* (9.3±0.9)	24±2* (32±1)	54±1* (48±1)	0.21±0.03 (0.19±0.05)	0.28±0.03 (0.27±0.02)
PAC (n=8)	96±4 (99±4)	112±4 (108±4)	33±1* (20±1)	6.4±0.5* (9.8±0.6)	26±1* (32±1)	55±4* (40±3)	0.33±0.08* (0.19±0.04)	0.26±0.01 (0.27±0.01)
Isoproterenol (n=6)	103±8 (101±6)	198±14* (110±4)	26±2* (19±1)	13.3±1.6* (9.6±1.1)	24±1* (30±1)	54±3* (48±2)	0.38±0.07* (0.21±0.02)	0.32±0.02* (0.26±0.01)
Propranolol (n=6)	103±3 (104±2)	100±3 (103±3)	15±1* (21±1)	7.7±0.9* (10.1±1.1)	36±2* (29±1)	40±2* (47±1)	0.21±0.04 (0.18±0.03)	0.28±0.02 (0.26±0.01)

Values are means ± SEM. Values in parentheses are respective baseline values. AoP, mean aortic pressure; HR, heart rate; RVP_{max}, right ventricular peak systolic pressure; SS, % segmental shortening; PvO_2, right coronary venous pO_2; O_2 Extr, oxygen extraction; GU, glucose uptake; LU, lactate uptake. * $p<0.05$ vs. untreated respective baseline.

Figure 1. Instrumentation

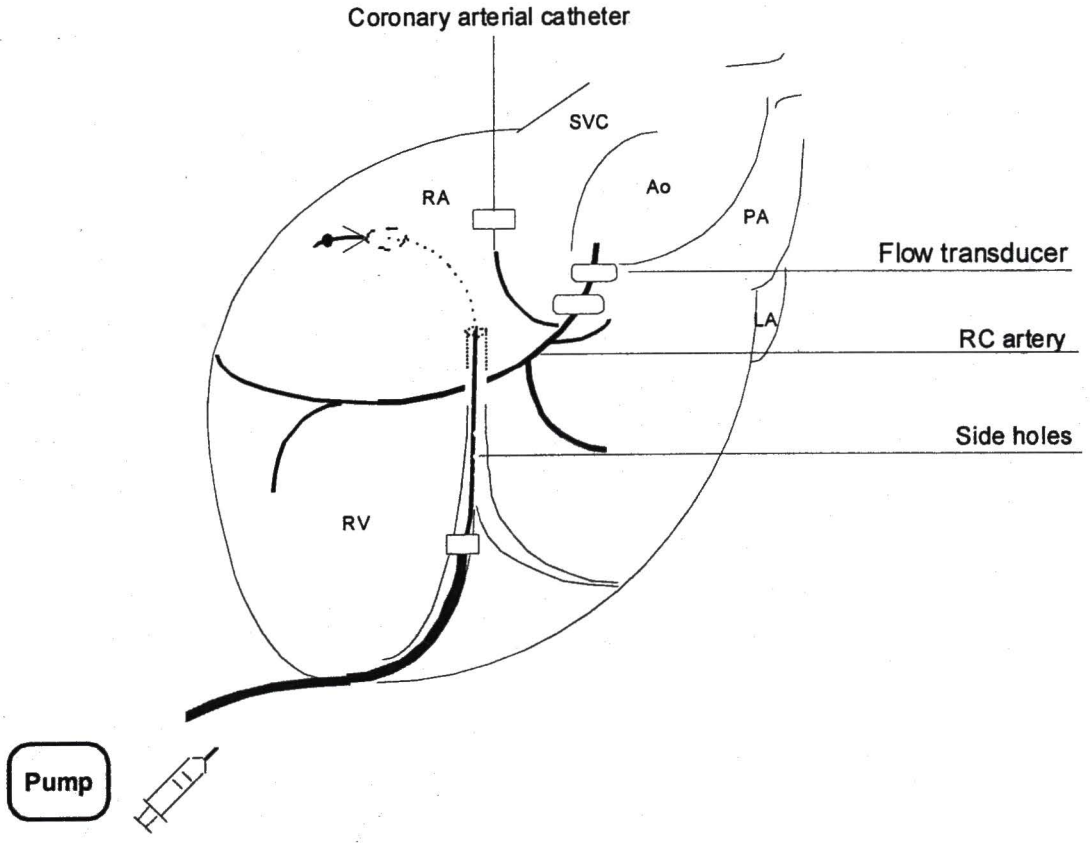

Figure 2. Oxygen Supply/Demand Balance During Changes in Demand

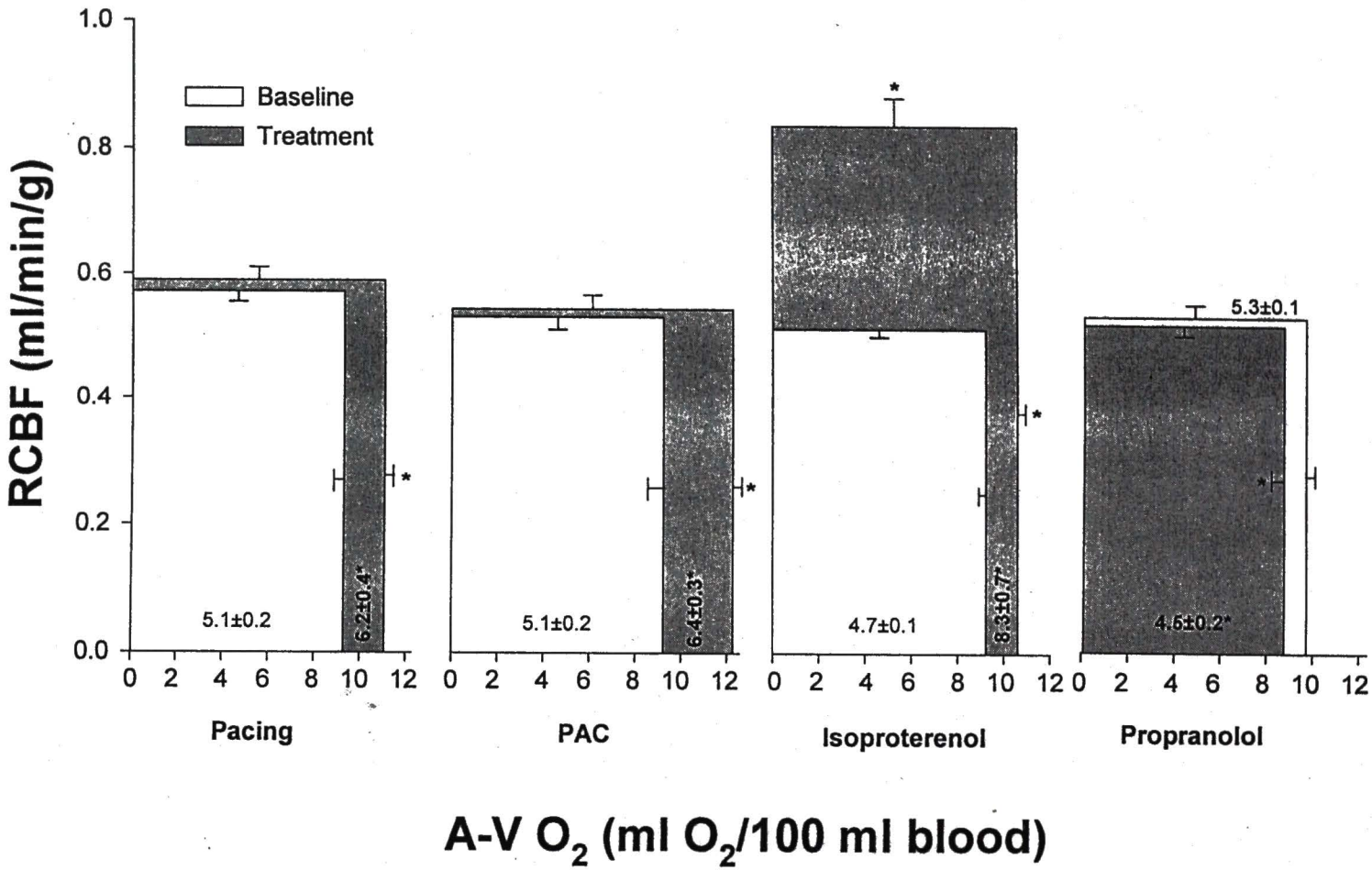

Figure 3. Relationship Between RV Oxygen Supply and Demand

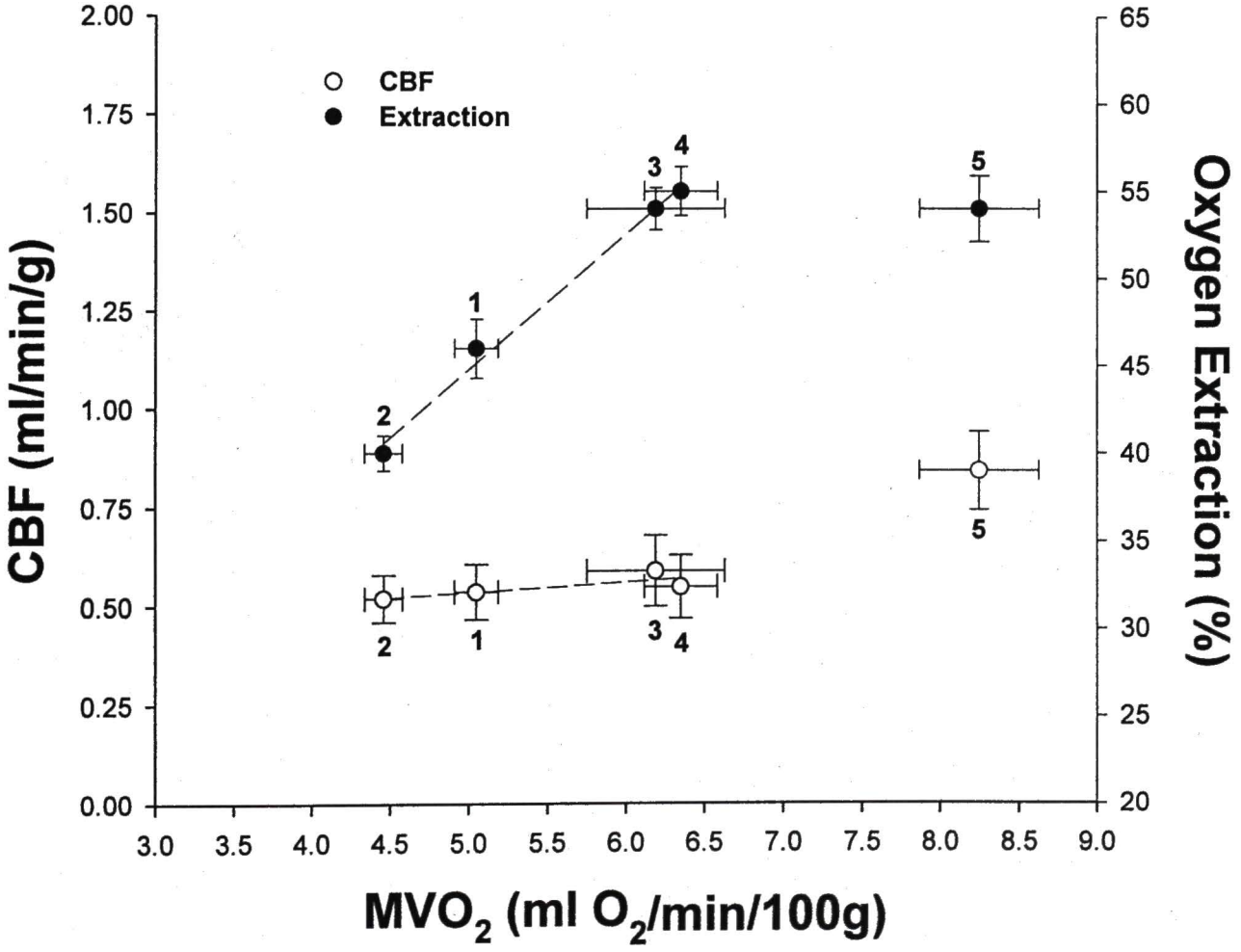

Figure 4. Relationship Between RV Coronary Resistance and Venous pO_2

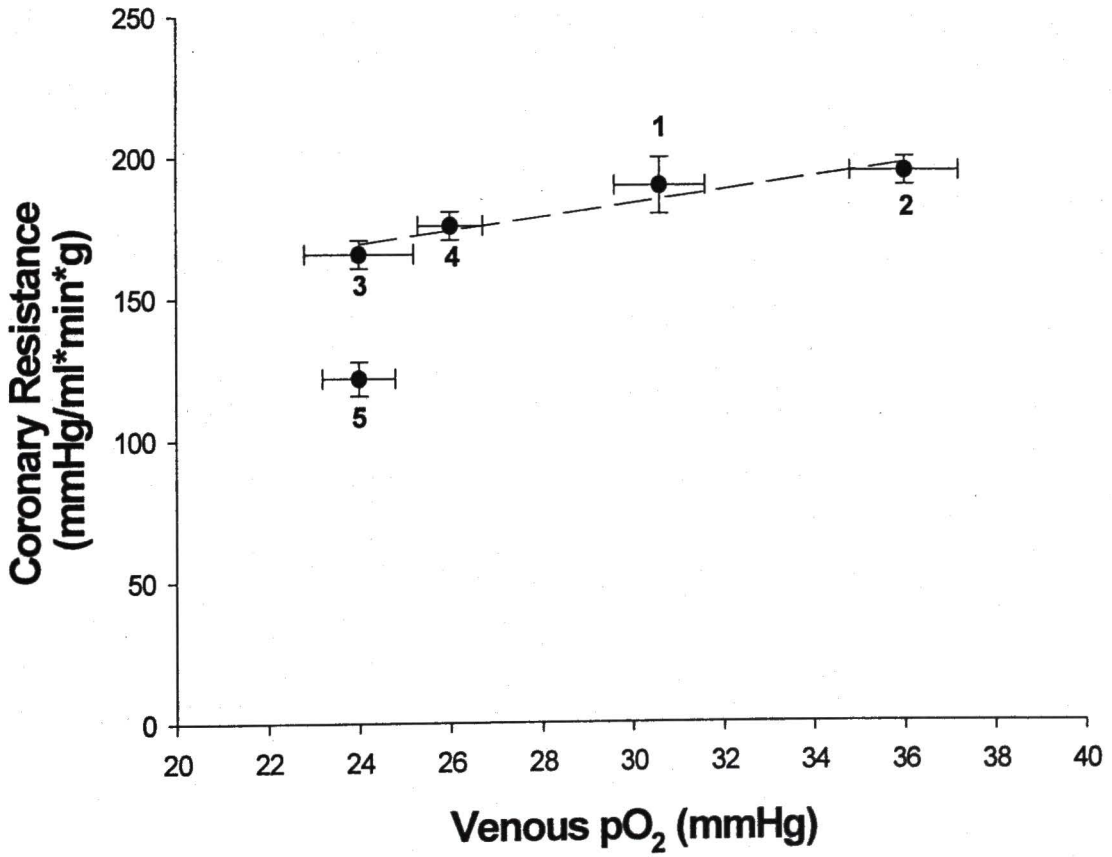

Figure 1. Instrumentation of the right ventricle. The right coronary venous catheter was inserted into a branch of the bifurcation and advanced proximally into the right atrium (RA). The proximal end was then sutured to the RA myocardium. The side holes of the venous sampling catheter were only located within the common superficial vein immediately proximal to the bifurcation. SVC, superior vena cava; Ao, aorta; PA, pulmonary artery; LA, left atrium; RV, right ventricle.

Figure 2. Oxygen supply/demand balance during changes in demand. The area of a given rectangle is the myocardial oxygen consumption (MVO_2, ml O_2/min/100g tissue), computed as arteriovenous O_2 content difference (x-axis) times the normalized right coronary blood flow (y-axis). Treatment data (shaded areas) are plotted with the respective baseline data (open areas). The shaded areas indicate MVO_2 associated with the different treatments; these values, and the respective baseline values, are labeled within the graph. PAC, pulmonary artery balloon catheter inflation. * $p<0.05$ vs. respective baseline values.

Figure 3. Relationship between RV oxygen supply and demand. CBF and O_2E are plotted as functions of MVO_2. The regression line (dashed line) indicates the relationship of these variables during interventions aimed at altering RV MVO_2. The numbers indicate the treatment conditions: 1, pre-treatment values; 2, propranolol bolus; 3, atrial pacing; 4, pulmonary artery constriction; 5,

isoproterenol infusion. Over the range of MVO_2 obtained by our treatments, the right ventricle relies upon increases in O_2E, as CBF was not altered without direct vasodilatory effects of the intervention.

Figure 4. Relationship between RV coronary resistance and venous pO_2. Coronary resistance is plotted as a function of venous pO_2. The numbers indicate the treatment conditions: 1, pre-treatment values; 2, propranolol bolus; 3, atrial pacing; 4, pulmonary artery constriction; 5, isoproterenol infusion. The dashed line indicates the line of regression, plotted with the exclusion of the isoproterenol data point. As the MVO_2 obtained by our treatments increased, the pO_2 of the venous blood fell accordingly, indicative of the increase in O_2E responsible for the increase in O_2 supply. Coronary resistance, however, was not substantially affected. It appears all points between 3 and 2 would fall near a linear relationship with slope slightly > zero. Point #5 deviates from this line, due to direct β_2-mediated vasodilation of vascular smooth muscle.

CHAPTER III

SYNOPSIS

To review, the first aim of this investigation was to determine the relative contributions of oxygen extraction and coronary flow reserves during acute increases in RV oxygen demand. The previous investigation resulted in new, informative data that suggests significant differences between the ventricles concerning the mechanisms regulating oxygen supply/demand balance. However, without RV MVO_2 values more similar to those measured in the LV, comparison of oxygen supply mechanisms employed by the two ventricles was not definitive. It was, therefore, beneficial to define the relative contributions of RV oxygen extraction and flow reserves to the increase in oxygen supply during higher levels of RV MVO_2. We decided to examine this topic in the exercising dog.

Substantial acute increases in RV oxygen demand were created using a submaximal exercise routine. We hypothesized that the RV oxygen extraction reserve would be utilized initially, followed by mobilization of the coronary blood flow reserve during graded treadmill exercise. We found that during the initial

lower level of exercise (Exer 1), RCBF and O_2E increased significantly. Two higher levels of exercise (Exer 2 and 3) resulted in increases in O_2E exclusively; RCBF was not affected. The highest level of exercise resulted in a further increase in O_2E, but RCBF was also substantially enhanced. The increase in RCBF upon initiation of exercise was almost exclusively due to the increase in mean aortic pressure; RC resistance was unaffected until the highest exercise level. In agreement with data presented in the preceding chapter, we found a large RV O_2E reserve under resting conditions. This reserve contributes preferentially to meeting RV O_2 demand during light and moderate exercise. Only at more intensive exercise is the substantial RC flow reserve mobilized. Furthermore, RC vascular resistance is not affected by large decreases in RC pO_2 until the RV O_2E reserve is exhausted. These findings, presented in the following manuscript, demonstrate that the RV and LV use different strategies to meet the increased myocardial oxygen demands of exercise over the range of oxygen demand associated with light to moderate exercise.

CHAPTER IV

MECHANISMS OF RIGHT VENTRICULAR OXYGEN SUPPLY/DEMAND BALANCE IN THE EXERCISING DOG

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Abstract

No data exist in the literature describing the myocardial oxygen supply/demand relationship of the right ventricle during exercise. A novel technique developed in our laboratory enables us to collect right ventricular (RV) venous blood samples from conscious dogs. RV oxygen supply/demand balance was examined in conscious dogs, chronically instrumented to measure right coronary blood flow (RCBF), segmental shortening (%SS), and peak systolic RV pressure (RVPmax) during increases in myocardial oxygen demand induced by four levels of dynamic exercise. The initial level of exercise created a 45% increase in RV myocardial oxygen consumption (MVO_2), commensurate with increased RVPmax, %SS, and heart rate ($p<0.05$). This change led to increases in oxygen extraction (O_2E) and RCBF ($p<0.05$). As RV MVO_2 increased further with two higher levels of exercise ($p<0.05$), the RV utilized its O_2 extraction reserve, as RCBF was not altered. At the highest level of exercise, a further 36% increase in RV MVO_2, created by significant increases in HR and RVPmax ($p<0.05$), resulted in a fall in RC resistance, and the flow reserve contributed more significantly to the oxygen supply. Therefore, the RV has a large O_2E reserve under resting conditions, and this reserve contributes preferentially to meeting RV O_2 demand during light and moderate exercise. Only at more intensive exercise is the substantial RC flow reserve mobilized. Furthermore, RC vascular resistance is not affected by large decreases in RC pO_2 until the RV O_2E reserve is exhausted. These findings demonstrate that the RV and LV use different strategies to meet the increased

myocardial oxygen demands of exercise over the range of oxygen demand associated with light to moderate exercise.

Key words: oxygen supply/demand balance, right ventricle, oxygen extraction, exercise

Introduction

The parameters of myocardial oxygen consumption (MVO_2), coronary blood flow (CBF), and percent oxygen extraction (O_2E) are key variables used to evaluate O_2 supply/demand relationships. MVO_2 reflects the oxygen demand of the myocardium when oxygen is not limited, and CBF and O_2E reflect the coronary vascular responses required to supply this oxygen demand. This study was designed to define contributions of RC flow and O_2E reserves, which are available to increase myocardial oxygen supply when RV oxygen demand is increased by graded treadmill exercise.

Numerous investigations have focused on cardiac hemodynamic and metabolic responses to dynamic exercise. In LV, the increase in MVO_2 during exercise always produces concomitant increases in coronary blood flow (1, 2, 10, 12, 13, 15, 34, 36). LV oxygen extraction is high at rest (14, 18, 22), and some studies have reported that left coronary (LC) venous pO_2 does not decrease during graded exercise (11, 12), although other studies noted small decreases (15, 27, 36). Thus, the LV oxygen extraction reserve is small, and it contributes minimally to enhancement of LV oxygen supply during increases in LV oxygen demand, such as produced by exercise. On the other hand, the RV has a large extraction reserve at rest (4, 14, 18, 22, 24), and this reserve might be mobilized to contribute significantly to RV oxygen supply during increases in RV oxygen

demand. The RV also has a large RC flow reserve (30, 37), and several studies have reported that RC flow increases during exercise (2, 19, 21, 26, 27). However, no investigations have investigated the relative roles of RV flow and oxygen extraction reserves in a conscious animal during acute alterations in oxygen demand.

The right ventricle (RV) generates a much lower systolic pressure than the left ventricle (LV), and consequently the RV has a lower oxygen demand. Few investigations have focused on right coronary (RC) vascular control and oxygen supply/demand balance, and, to date, no investigations have reported on these issues in a conscious model. Recently our laboratory developed a procedure to obtain RC venous samples from conscious dogs (5). Measurements of O_2 content in these samples and in arterial blood enable RV oxygen demand to be determined. This procedure was used to investigate RV oxygen balance in exercising dogs.

In this investigation, we observed that the RV oxygen extraction reserve was mobilized as exercise progressed, and this reserve contributed importantly to RV oxygen supply during exercise. RC flow initially increased along with an increase in aortic blood pressure, but only during strenuous exercise did RC vascular resistance fall. In addition to defining contributions of RC flow and RV oxygen extraction reserves to RV oxygen supply during exercise, these findings

demonstrate that RC resistance is insensitive to large changes in venous oxygen tension, at least above a critical threshold. When these results are compared with published LV data, important differences in mechanisms of ventricular oxygen balance are revealed.

Materials and methods

Animal instrumentation

This investigation was approved by the Institutional Animal Care and Use Committee and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1996). Eight adult mongrel dogs of either sex, weighing 24 to 32 kg were studied. Thirty minutes after preanesthesia treatment with PromAce (0.03 mg/kg, *i.m.*), anesthesia was induced by thiopental sodium (5 mg/kg, *i.v.*). Following endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with isoflurane gas (1-3%) with equal offset of oxygen (1 L). Under sterile conditions, a thoracotomy was performed in the fourth right intercostal space, and the dog was instrumented as illustrated in Fig 1. A Tygon catheter (1.0 mm i.d., 1.8 mm o.d.) was inserted into the aorta through the right internal mammary artery to measure aortic blood pressure. A Konigsberg model P6.5 pressure transducer was inserted through a stab wound in the RV infundibulum and secured with a purse string suture. A nonbranching section of the right coronary artery (RCA) was dissected free for 1 - 2 cm to affix a Transonic® flowmeter. A coronary venous catheter prepared from Micro-Renathane tubing (type MRE-025, 0.31 mm i.d., 0.64 mm o.d.) was inserted into a superficial vein draining the RV myocardium, as described previously (5). To measure RV segment shortening, a pair of piezoelectric crystals was inserted into the mid wall of the RV. These

crystals were positioned 1 cm apart and perpendicular to the main right coronary artery.

After instrumentation, catheters and wires were brought out of the thorax through the 3rd and 5th right intercostal spaces, tunneled under the skin, and exteriorized between the shoulders through individual puncture wounds. The chest was closed, and the pneumothorax evacuated through a chest tube. Antibiotic (Clavamox®, 6.25 mg/lb, b.i.d., p.o.) and aspirin (162 to 300 mg, p.o.) were given for 10 days after surgery. The RC venous catheter was attached to an Access Technologies "C" Series® Balloon Pump (60 ml volume elastomeric pump, 2.0 ml/hour flow rate) for continuous infusion of heparinized saline (10 units/ml) for the duration of experimentation. The RC catheter was flushed with heparinized saline (10 units/ml) and filled with heparin (5000 units/ml) daily. Other catheters were treated similarly at three-day intervals.

Data collection

After recovery from surgical procedures, measurements were obtained with the animal standing quietly on a treadmill. RC flow was measured with a Transonic® T106 series flowmeter. A pressure transducer (Narco Telecare® model LDI-5) was positioned at mid-heart level and connected to the mammary artery catheter to measure aortic blood pressure (AoP). Ultrasonic signals from length dimension crystals were processed by a Triton Technology model 120

sonomicrometer and monitored with a Tektronics model 2215A oscilloscope. Segment lengths at the beginning of the positive deflection of the dP/dt record were considered end diastolic, and those measured 20 ms before the peak negative deflection were considered end systolic, as previously described (35). RC flow, right ventricular pressure (RVP), dP/dt, AoP, and RV segment length were recorded on a multichannel Coulburn® chart recorder. Data were then extracted by hand from the recordings. Segment shortening was calculated as a percent by the formula: {%SS = (end diastolic length – end systolic length) / (end diastolic length)*100}. Right coronary resistance was calculated by dividing mean AoP by RCBF. Oxygen extraction was determined by the formula: {O_2E = [(O_2 $Content_{arterial}$ - O_2 $Content_{venous}$) / O_2 $Content_{arterial}$] * 100}. During analysis of the Exer 1 and Exer 4 data, we wanted to determine the relative contributions of RCBF and A-V O_2 content difference to the observed increase in RV oxygen consumption. It was determined that the percent contribution of each variable was directly proportional to the percent increases from values at the preceding exercise level.

Plasma Sample Collection and Analyses

Arterial and RC venous blood samples were collected to determine oxygen content, pO_2, and glucose and lactate concentrations. All samples were collected anaerobically and chilled on ice until analyses. Oxygen content and pO_2 were determined by an Instrumentation Laboratory® Co-Oximeter and a

Synthesis 30 Blood Gas Analyzer, respectively. Arterial oxygen content was not significantly affected by exercise (control 18.0±0.6 vs. post-exercise 18.9±0.9 ml O_2/100 ml blood, p=0.459). MVO_2 was determined by multiplying the arteriovenous difference of oxygen content by the coronary blood flow, normalized per gram tissue mass. Blood glucose and lactate concentrations were determined by a Yellow Springs Instruments® Model 2300 STAT Plus Glucose and L-Lactate Analyzer. Glucose and lactate uptakes were calculated by multiplying the arteriovenous substrate difference by the normalized coronary blood flow.

Exercise protocol

A standardized submaximal exercise protocol was used (34). Baseline measurements were taken with the dog resting quietly on the treadmill, then exercise was begun with a 3-mph warm-up period for three minutes. The speed of the treadmill was then increased to 4 mph for the first level of exercise (Exer 1). This treadmill speed was continued for the remainder of the experiment. The second level of exercise (Exer 2) was created by increasing the incline of the treadmill to 4%. Further increases in treadmill incline to 8 and 16% were defined as Exer 3 and 4, respectively. The animal was exercised for three minutes at each level. Blood samples were taken and measurements recorded during the last minute of each level.

Measurement of RCA Perfusion Territory

After termination of the experiments, the animal was euthanized with sodium pentobarbital (30 mg/kg, i.v.) followed by potassium chloride. After opening the chest, approximately 15 ml of 2.5% Evan's Blue dye was injected with a syringe into the right coronary arterial catheter to delineate the perfused territory. This territory was then carefully excised and weighed to normalize the coronary flow per gram of tissue mass.

Statistical Analysis

All values are expressed as means ± SEM. Results were analyzed with one-way repeated measures (within subject design) analyses of variance (ANOVA). When significance was found ($p<0.05$), a Student-Newman-Keuls multiple comparison test was performed. Analyses were performed and interpreted according to Keppel (17) and Zar (38). Statistical procedures were performed using Sigma Stat Statistical software version 2.0.

Results

Table 1 presents hemodynamic and metabolic data collected at rest and during the exercise protocol. Mean aortic blood pressure significantly increased by 19% at the first exercise level, but was not further altered by higher exercise intensity. Heart rate increased significantly during the first exercise level and increased further during the third and fourth levels. Peak systolic RVP increased during the first exercise level, and then increased further at the highest level of exercise.

Right coronary blood flow increased during the first level of exercise, but was not further altered during the second and third exercise levels. At the highest level of exercise (Exer 4), RCBF increased sharply over the Exer 3 value (Fig 2). RC venous blood pO_2 fell 20% during the initial exercise level, and fell progressively with each level of increased exercise intensity (Table 1). Oxygen extraction increased significantly upon the initiation of exercise, and increased further at each successive exercise level (Fig 2). Exercise stimulated glucose uptake during the first three exercise levels, and this uptake was further enhanced during the fourth level. Lactate uptake was unaffected by exercise, until the highest level, where this uptake was significantly diminished. Regional contractile function (%SS) was higher than resting values at every exercise level, but did not vary significantly between exercise levels.

Figure 2 provides information describing the oxygen supply/demand balance of the RV during exercise-induced increases in oxygen demand. With the A-V O_2 content difference on the x-axis and the normalized RC blood flow on the y-axis, the area within the rectangle represents RV MVO_2. Analysis of this data allowed us to determine the contributions of mechanisms responsible for increasing RV oxygen supply to meet demand. During the initial 45% increase in MVO_2 at the first exercise level, both RCBF and the A-V O_2 content difference significantly increased; the increase in O_2E provided 57% of the incremental increase in RV MVO_2, and the increase in RCBF provided 43%. As MVO_2 further increased by 20% at Exer 2 and by 19% during Exer 3, RCBF was not affected. Therefore, these increases in RV MVO_2 were provided entirely by increases in O_2E. During the highest level of exercise, the incremental increase in RV MVO_2 was 36%. RCBF increased 42%, and the O_2E content difference increased 11%. Thus, the increase in RCBF was responsible for meeting 79% of the incremental increase in RV oxygen demand, and the increase in O_2E was responsible for 21% during this final step increase in exercise intensity.

The data collected in the present study were compared with LV data reported earlier by plotting oxygen supply variables as functions of oxygen demand, i.e., MVO_2 (Figs 3 and 4). This approach normalizes for differences between experiments that impact oxygen demand, such as afterload, heart rate, and contractile state of the myocardium (36). RCBF increased significantly with

the initial 45% increase in MVO_2 (see Fig 3), and this increase was due exclusively to a concomitant increase in aortic pressure at the onset of exercise (Table 1). Further moderate increases in RV MVO_2 did not alter CBF. Thus, there is no relationship between CBF and MVO_2 over this range (slope ~ 0). Higher intensity exercise resulted in an increase in RCBF. Conversely, LCBF increases linearly with increases in LV MVO_2.

A similar approach was utilized to plot venous pO_2 against MVO_2 (Fig 4). RV venous pO_2 decreased linearly over a large range as RV MVO_2 increased during exercise. Conversely, venous pO_2 of the LV does not decrease substantially with large increases in LV MVO_2. At the highest level of exercise, RV MVO_2 was similar to LV MVO_2 during light exercise; RV and LV venous pO_2 were similar.

Coronary resistance was calculated by dividing the driving pressure (AoP) by the observed CBF, and this variable is plotted against MVO_2 in Fig 5. The lines indicating the proposed relationships of the left and right ventricles were visually approximated. RC and LC resistances responded differently during low intensity exercise, since RC resistance fell slightly and LC resistance fell abruptly over this range of MVO_2. The shaded area in Fig 5 reflects the greater RV O_2E reserve, which is mobilized before RC resistance falls to values measured in the LV.

RC and LC resistances are plotted against RC and LC venous pO_2 (Fig 6). As the venous oxygen tension declined with incremental increases in oxygen demand, RV vascular resistance was unaffected by changes in venous pO_2 above 20 mmHg. At this pO_2, further reductions resulted in a decline in coronary resistance. The gray area represents the operating range of the LV. In the LV, with basal venous pO_2 values at or below 20 mmHg, any decline in pO_2 was associated with a concomitant fall in LV coronary resistance. The line indicating the proposed relationship of the left and right ventricles was visually approximated. Coronary venous pO_2 of 20 mmHg appears to be a threshold for oxygen related changes in coronary resistance; however, without LC values greater than 20 mmHg, we cannot be certain that this threshold is the same for both ventricles.

Discussion

This report describes the first investigation of RV oxygen supply/demand balance during exercise. Although LV oxygen supply mechanisms have been investigated extensively in exercising animal models (1, 11-13, 15, 20, 36), RV mechanisms have not been investigated, apparently due to the difficulty of collecting RC venous blood samples from conscious animals. This difficulty is due primarily to the small size and fragility of the superficial veins draining the RV and the lack of a common drainage area, such as the coronary sinus. We recently developed a procedure for collecting RC venous blood samples from conscious dogs (5). Utilizing this procedure, we found: 1) The RV has a large O_2E reserve under resting conditions, and this reserve contributes preferentially to meeting RV O_2 demand during light and moderate exercise. Only at more intensive exercise is the substantial RC flow reserve mobilized. 2) RC vascular resistance is not affected by large decreases in RC pO_2 until the RV O_2E reserve is exhausted. These findings demonstrate that the RV and LV use different strategies to meet the increased myocardial oxygen demands of exercise.

Myocardial oxygen supply is a function of coronary blood flow and oxygen extraction, i.e., the percentage of the arterial oxygen that is removed from the blood. LV resting O_2E is nearly maximal, with reported values as high as 79% (13, 18, 30, 33, 36). Thus, changes in LV oxygen demand must be met primarily

by altering LC flow. In contrast, RV resting O_2E is only about 44% (16, 18, 24), so the RV has a large oxygen extraction reserve as well as a substantial flow reserve (7, 18, 19, 24). Both of these reserves are potentially available to supply oxygen when RV oxygen demand increases, as during exercise. Studies in anesthetized dogs have demonstrated that the RV O_2E reserve can be mobilized to meet increased RV oxygen demand produced by pacing, isoproterenol infusion, or pulmonary artery constriction (18, 28-30). However, anesthesia, open-chest surgery, and perfusion systems may have blunted RC vasoconstrictor tone in these experiments (6, 8, 9, 31), and, thus, yielded inappropriately high RC flow and low RV O_2E measurements. Experiments in conscious dogs were required to ascertain that RV O_2E reserve is, indeed, greater than that of the LV, and then to define the extent to which this reserve contributes to RV oxygen supply during exercise.

Our measurements of resting RV O_2E in conscious dogs agree closely with values reported previously for anesthetized dogs. With resting RC flow of 0.55±0.03 ml/min/g and O_2E of 43±2%, and assuming a potential increase in O_2E to 80%, as observed in the LV of exercising dogs (13, 36), this increase in O_2E reserve could contribute an additional 3.7 ml O_2/min/100 g with no increase in flow. At the exercise levels studied, RV MVO_2 increased from 5.1±0.2 at rest to 14.4±1.0 ml O_2/min/100 g at the most intensive exercise. Thus, the RV O_2E reserve might have supplied 40% of this increase in RV oxygen demand. At the

less intensive exercise levels of this study, the O_2E reserve could have supplied the entire increase in RV MVO_2. Our measurements showed that increased O_2E alone contributed 41% of the increase in RV MVO_2 at the highest exercise level and a greater percentage of required oxygen during more moderate exercise. Clearly, the RV O_2E reserve is an important factor in RV oxygen supply/demand balance.

RC blood flow, the other determinant of RV oxygen supply, has been measured in exercising dogs (2, 26), horses (21), and ponies (19, 27). These studies have all reported increases in RC flow, as we observed at our most strenuous exercise. Thus, these earlier studies have implied no differences in RC and LC flow responses to exercise, i.e., the RC flow reserve is mobilized to meet increasing RV demands for oxygen. Of course, these studies did not investigate contributions of O_2E.

It is difficult to compare results of earlier RC flow studies with our findings, since RV MVO_2 was not measured and, thus, the degree of oxygen demand during exercise cannot be readily equated. However, in an earlier canine study, Ball et al. (2) measured RC flow with radioactive microspheres during graded treadmill exercise. Their resting flows were similar to ours, but with each increment in heart rate, their flows greatly exceeded those we observed at comparable heart rates. For example, the flow reported by Ball et al. for

moderate exercise (HR = 185±2) was 2.8 times our measured flow at Exer 3 (HR = 191±5). Ball et al. also measured LC flows and concluded that RC and LC flows increase at similar rates during exercise. Our findings of little change in RC flow during mobilization of the O_2E (Fig 2) clearly differ from the conclusions of Ball et al. The discrepancy is unlikely to be due to different measurement techniques since resting RC flows are similar in both studies. One difference is the time of measurement. In our protocol, data were collected at three minutes of exercise at each level, whereas Ball et al. injected microspheres at 45 s, a time when heart rate had stabilized, but perhaps not RC flow. In fact, we observed transient increases in RC flow during protocol steps that required 60-84 s to subside. It is also possible that the dogs of Ball et al. had a lesser O_2E reserve, and, therefore, had to mobilize their flow reserve to a greater degree. Once the O_2E reserve is exhausted, our data (Fig 2) do agree with Ball et al.'s suggestion that RC flow increases in parallel with LC flow.

Substrate selection

In this investigation, RV glucose uptake was enhanced during exercise, in agreement with findings of other LV investigations (3, 23). As arterial lactate concentrations rise during dynamic exercise, it is generally accepted that lactate uptake increases, as long as oxygen is available (25). In our investigation, however, arterial concentrations were not significantly altered over the brief 12

min period, and lactate uptake did not increase. During the highest exercise level, glucose uptake was further enhanced and lactate uptake decreased.

Regional function, heart rate, and RV MVO_2

During the initial exercise level, %SS increased 56% and HR increased 61%. The concomitant 45% increase in RV MVO_2 was a result of increases in both variables. Further increases in exercise intensity did not alter %SS as HR increased another 35%. The resultant increase in RV MVO_2 during the highest level of exercise intensity was, therefore, not due to enhanced myocardial contractile shortening.

Validation that RC venous samples reflect RCA drainage

For our conclusions to be valid, our RC venous blood samples must contain only blood draining RV myocardium supplied by the RCA. Two possible sources of contamination are blood from the right atrium and blood originating from non-RCA nutritive vessels. To investigate whether there was contamination from right atrial blood, radioactive microspheres were infused simultaneously for 5 min into the superior and inferior vena cavae of 4 dogs (three were instrumented and studied in the conscious state and one was studied in an acute experiment). During infusion of radioactive microspheres, blood samples were collected from the right atrium and the RC vein and later analyzed for radioactivity. Since the microspheres were trapped within the pulmonary

circulation, any radioactivity within the RC venous samples would have come from right atrial contamination. Mean radioactivity counts emitted by the blood samples were 2636±702 for right atrial blood and 2±1 for RC venous blood samples. These data demonstrate that there was no right atrial blood withdrawn into the venous samples using our technique.

It is also possible, given the vascular anatomy of the right heart, that blood from the LV circulation may have contributed significantly to RV venous samples. This possibility of venous contamination was explored in an earlier study in our laboratory by Murakami et al. (24). They infused Evan's blue dye systemically, while perfusing the RCA from an uncontaminated blood supply. With RC perfusion pressure reduced to 80 mmHg and with normal systemic arterial pressure, the LC contribution to RC venous drainage was only 1.2±1.0 %. In the present study, there was no disparity between RC and LC perfusion pressures, so RC venous contamination from other coronary sources should have been negligible.

Differences in right and left ventricular oxygen supply/demand balance

This investigation produced data describing the relationship between RC blood flow, O_2E, venous pO_2, and RV MVO_2 during exercise. Similar data for the LV of conscious dogs have been recently reported by Tune et al. (36). Right and left heart data are compared in Figs 3-6. Fig 3 demonstrates that the relationship

between RC flow and RV MVO_2 diverges from that of the LV at rest and at lower MVO_2 when the RV O_2E reserve is still large. In the LV, with its small O_2E reserve, relatively small changes in venous pO_2 occur as MVO_2 increases (Fig 4). In the RV, venous pO_2 decreases greatly as MVO_2 increases, and the RV O_2E reserve is mobilized with little change in RC flow. Figs 3 and 4 demonstrate that the RV initially uses its O_2E reserve when RV oxygen demand increases, whereas the LV uses its flow reserve.

Coronary resistance can be estimated by dividing the driving pressure (AoP) by the observed coronary blood flow. This index is plotted against RV and LV MVO_2 during exercise in Fig 5. To increase LC flow, LC resistance is reduced during exercise by vasodilatory mechanisms that are still not understood (32). Conversely, we found the RC resistance falls only during the highest level of exercise. The difference in the two resistance curves plotted in Fig 5, demarcated by the shaded area, reflects mobilization of the RV O_2E when LV oxygen demand was sufficient to cause LC vasodilation.

Insight into the influence of myocardial oxygen tension, as reflected by coronary venous pO_2 (32, 36), on coronary resistance can be obtained by plotting RC and LC resistances vs. venous pO_2 (Fig 6). The shaded area demarcates the operating range for the LV; RV venous pO_2 is much greater at rest and during moderate exercise. For RV venous pO_2 above 20 mmHg, large

decreases in venous pO_2 had no effect on RC resistance. However, the graph shows that vascular resistance in both the RV and LV begins to decrease when ventricular venous pO_2 falls below 20 mmHg. If myocardial venous pO_2 is a direct or indirect controller of coronary resistance, these data demonstrate a threshold effect at about 20 mmHg venous pO_2 that is similar for both ventricles despite differing MVO_2 (Fig 4). These data should be helpful in further delineating the role of myocardial oxygen tension in the regulation of coronary resistance.

Summary

This report presents the first data describing RV oxygen supply/demand balance during graded exercise. The results demonstrate a substantial RV oxygen extraction reserve that is utilized preferentially during exercise-induced increases in RV MVO_2. Although RC flow increases during moderate exercise, this is primarily due to increases in blood pressure; RC resistance is not affected until the RV O_2E reserve is exhausted. In addition to defining the relative contributions of the RC flow and O_2E reserves to supplying the increased RV MVO_2 during exercise, we found that RC resistance appears to be less sensitive to changes in RC venous pO_2 than the LV, at least above a certain threshold venous pO_2. Considerable differences exist between the ventricles regarding the strategies used to match myocardial oxygen supply with demand.

Acknowledgements

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Table 1. Hemodynamic and metabolic data during exercise (n=8).

	AoP (mmHg)	HR (beats/min)	RVP_{max} (mmHg)	SS (%)	PvO_2 (mmHg)	O_2 Extr (%)	GU (μmol/min/g)	LU (μmol/min/g)
Rest	99±2	105±5	21±1	9.4±1.0	32±2	43±2	0.17±0.02	0.27±0.02
Exercise 1	118±3*	169±5*	30±2*	14.7±2.3*	25±2*	66±3*	0.31±0.03*	0.24±0.02
Exercise 2	120±4*	178±4*	31±2*	15.1±2.1*	23±1*†	69±4*†	0.34±0.03*	0.25±0.03
Exercise 3	118±7*	191±5*†	30±2*	13.5±1.6*	20±1*†	73±3*†	0.35±0.04*	0.24±0.03
Exercise 4	124±5*	228±6*†	37±3*†	11.5±2.8*	17±1*†	81±4*†	0.47±0.07*†	0.16±0.02*†

Values are means ± SEM. AoP, mean aortic pressure; HR, heart rate; RVP_{max}, right ventricular peak systolic pressure; SS, % segmental shortening; PvO_2, right coronary venous pO_2; O_2 Extr, oxygen extraction; GU, glucose uptake; LU, lactate uptake. † $p<0,05$ vs. previous exercise level, * $p<0.05$ vs. baseline.

Figure 1. Instrumentation

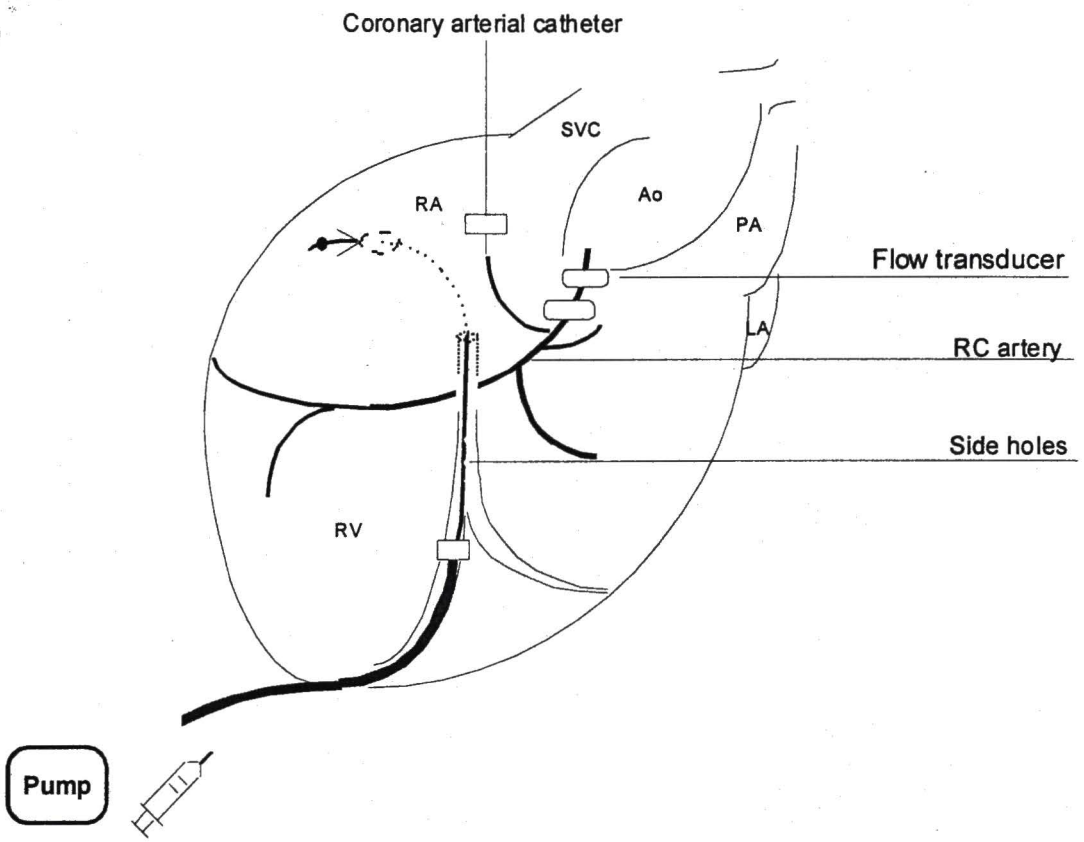


Figure 2. Oxygen Supply/Demand Balance During Graded Exercise

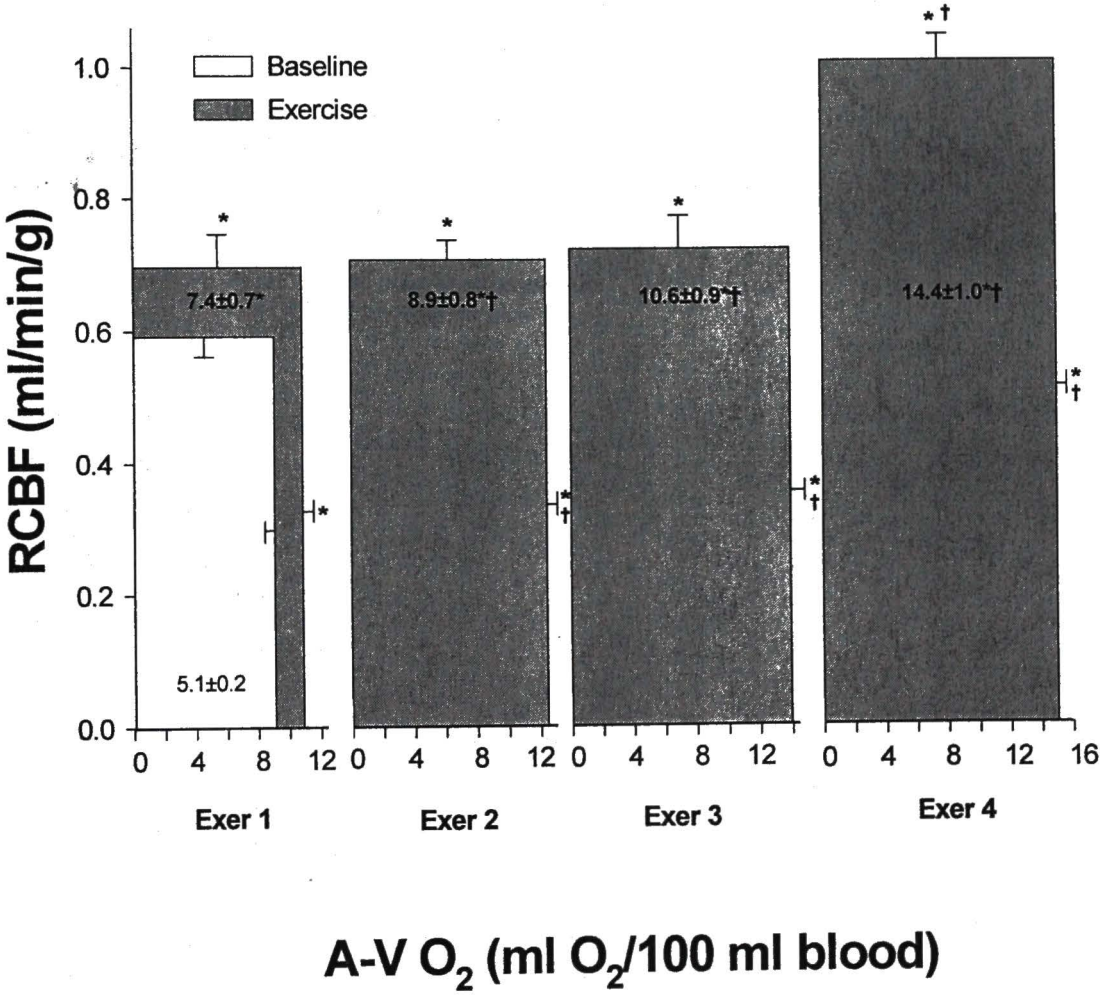


Figure 3. CBF/MVO_2 Relationship of the Right and Left Ventricles

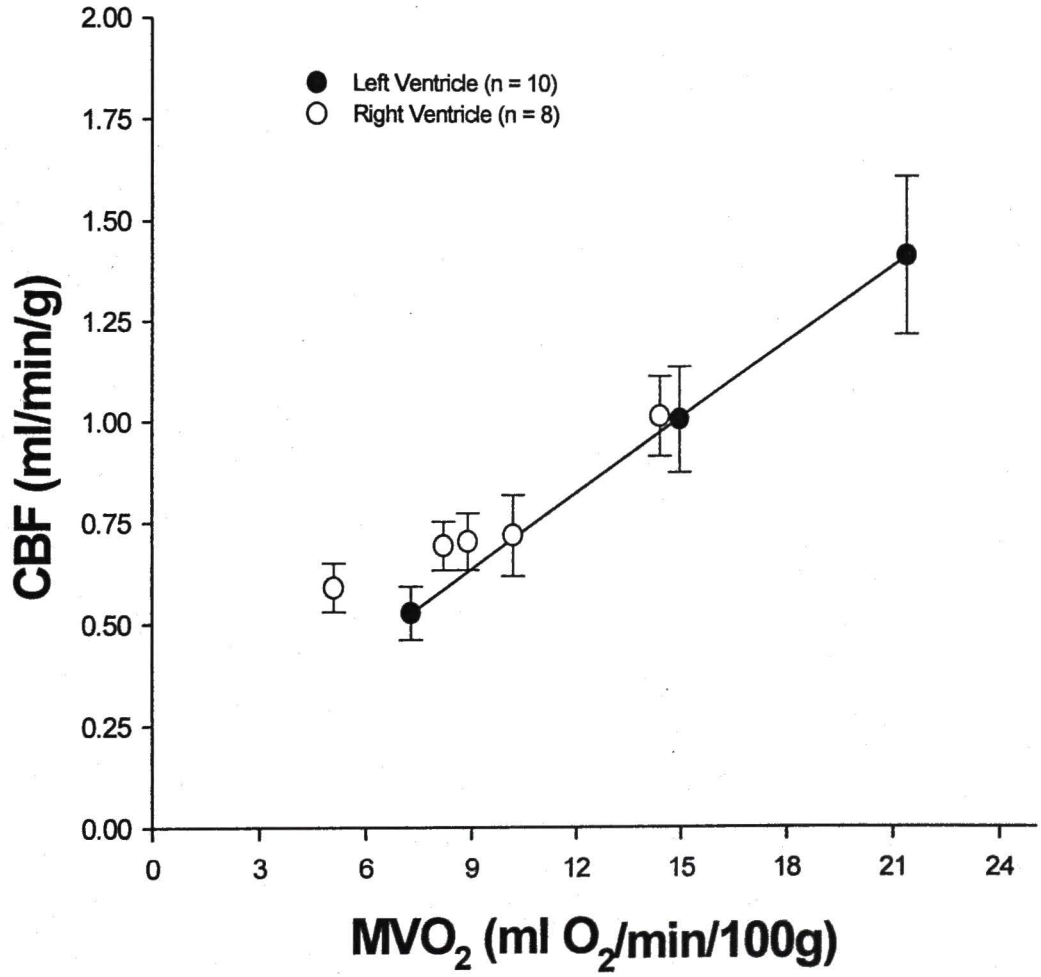


Figure 4. Venous pO_2/MVO_2 Relationship of the Right and Left Ventricles

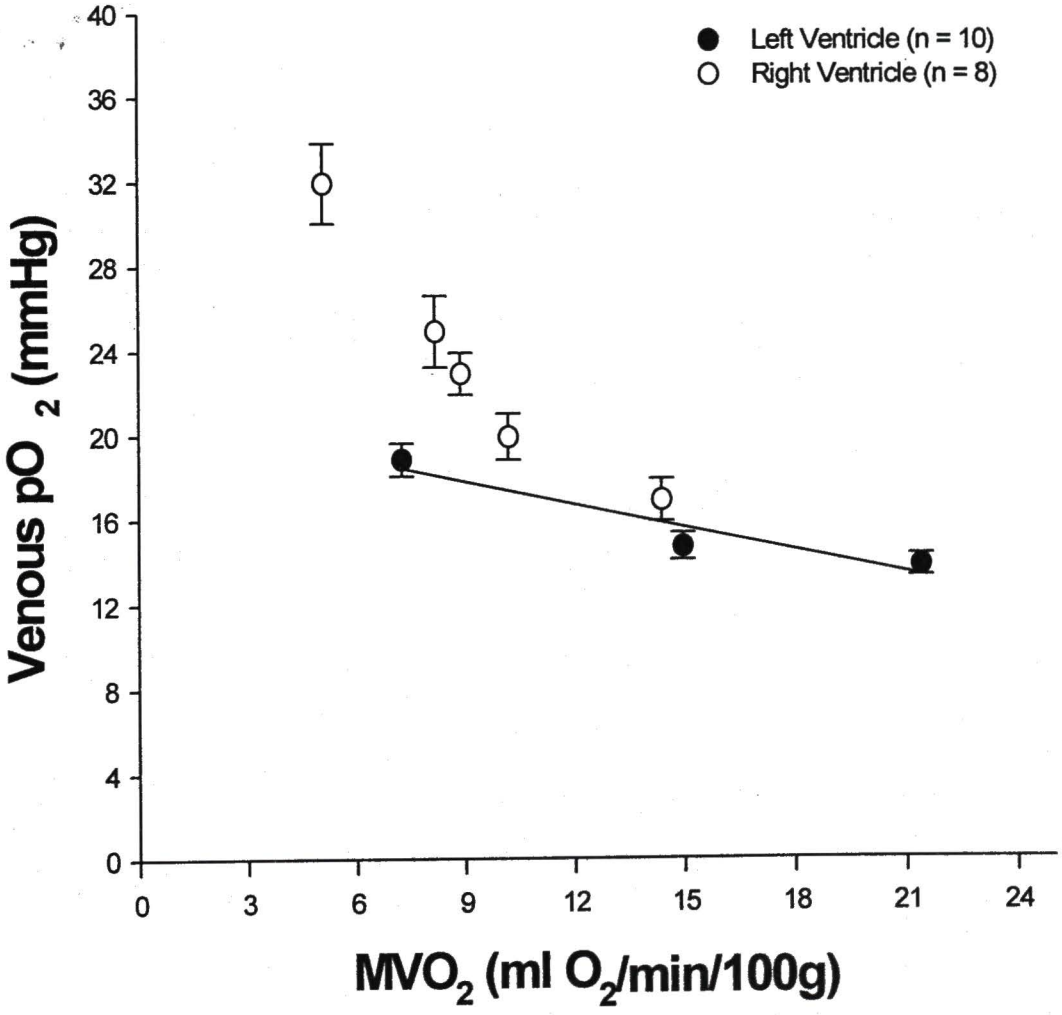


Figure 5. Relationship Between Coronary Resistance and MVO_2 of the Left and Right Ventricles

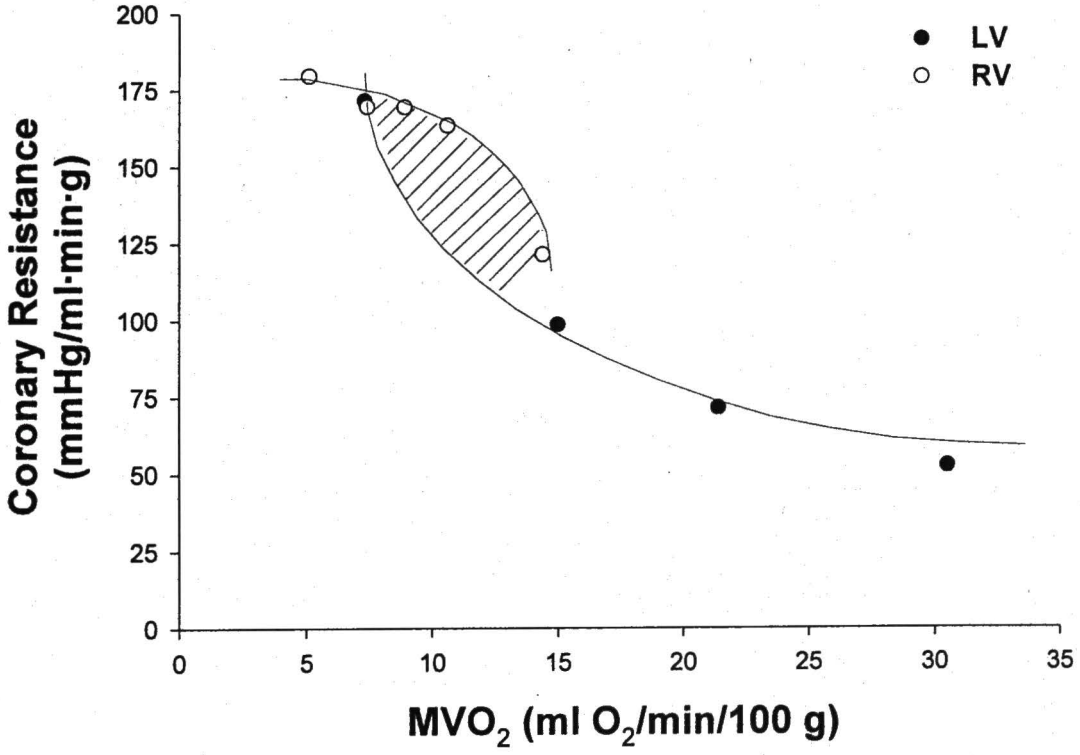


Figure 6. Relationship Between Coronary Resistance and Venous pO_2 in the Right and Left Ventricles

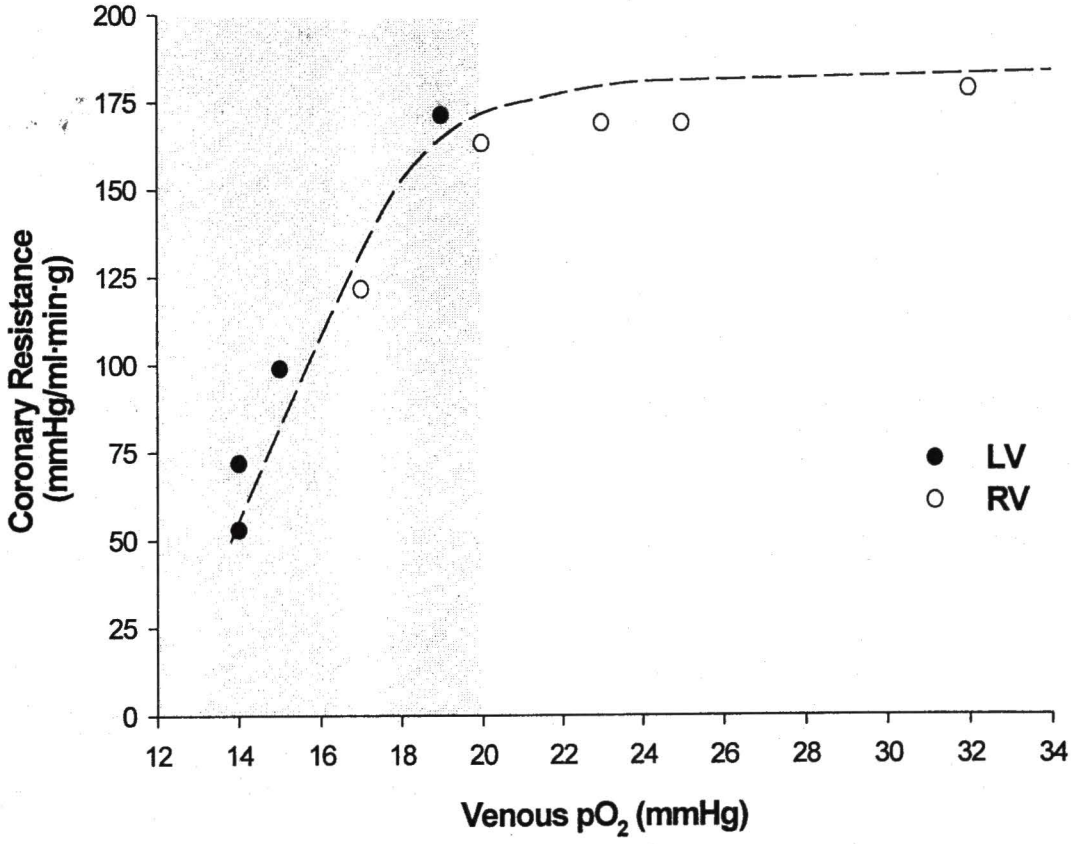


Figure 1. Instrumentation of the right ventricle. The right coronary venous catheter was inserted into a branch of the bifurcation and advanced proximally into the right atrium (RA). The proximal end was then sutured to the RA myocardium. The side holes of the venous sampling catheter were only located within the common superficial vein immediately proximal to the bifurcation. SVC, superior vena cava; Ao, aorta; PA, pulmonary artery; LA, left atrium; RV, right ventricle.

Figure 2. Oxygen supply/demand balance during exercise. The area within a rectangle is the myocardial oxygen consumption (MVO_2, ml O_2/min/100 g tissue) during that condition, as calculated by the arteriovenous O_2 content difference (x-axis) multiplied by the normalized right coronary blood flow (y-axis). Exercise data (gray areas) are plotted along with the respective baseline data (open area). The gray areas indicate increases in MVO_2 associated with the different exercise levels; these values, and the baseline value, are labeled within the graph. As RV MVO_2 increased from Exer 1 to Exer 3, the right ventricle relied upon increases in O_2E, as CBF was not altered. Further increases in MVO_2 at the highest level of exercise apparently exhausted the oxygen extraction reserve, necessitating an increase in RCBF. * $p<0.05$ vs. respective baseline values, † $p<0.05$ vs. previous level of exercise.

Figure 3. CBF/MVO_2 relationship of the right and left ventricles. CBF is plotted as a function of MVO_2. Data from the left ventricle collected during exercise and reprinted with permission (36). The regression line indicates the MVO_2/CBF relationship of the left ventricle.

Figure 4. Venous pO_2/MVO_2 relationship of the right and left ventricles. Venous pO_2 is plotted as a function of MVO_2. Data from the left ventricle collected during exercise and reprinted with permission (36). The regression line is drawn through the LV data. As the MVO_2 increased up to Exer 3, the pO_2 of the right ventricular venous blood fell accordingly, indicative of the increase in O_2E responsible for the increase in O_2 supply.

Figure 5. Relationship between coronary resistance and MVO_2 of the left and right ventricles. An index of coronary resistance (AoP/CBF) is plotted against MVO_2. Data from the left ventricle collected during exercise and reprinted with permission (36). Without data from an intermediate MVO_2 of the left ventricle (between about 7 and 15 ml O_2/min/100 g), the relationship is not complete. The hypothesized relationships are visually approximated. There appears to be a discrepancy between these relationships of the ventricles, and the shaded area represents the increased RV extraction reserve available with moderate MVO_2.

Figure 6. Relationship between coronary resistance and venous pO_2 of the left and right ventricles. An index of coronary resistance (AoP/CBF) is plotted against venous pO_2. Data from the left ventricle collected during exercise and reprinted with permission (36). The coronary resistance falls as venous pO_2 decreases below a certain level (about 20 mmHg). The gray area represents the range of LV values, as coronary sinus pO_2 over 20 mmHg is not observed under normal conditions.

CHAPTER V

SUMMARY AND CONCLUSION

This investigation was designed to delineate, for the first time, mechanisms responsible for maintaining oxygen supply/demand balance in RV myocardium of a conscious animal. RV oxygen demand was altered physiologically, by exercise and atrial pacing, and pharmacologically, by beta-adrenergic stimulation and blockade. The determinants of oxygen supply, O_2E and RC blood flow, were measured. Results of this investigation demonstrated that the RV O_2E reserve is mobilized initially when RV oxygen demand is increased, and only when this reserve is exhausted does RC resistance decrease. With a decrease in RV oxygen demand, O_2E falls with no change in RC resistance. These RV responses differ from those reported for LV, which has a much smaller O_2E reserve and, thus, varies its blood flow to meet changing requirements for oxygen.

One of the primary findings of this dissertation research is that in the conscious dog basal RV oxygen extraction is substantially lower than that of the

LV. This indicates that the CBF/MVO_2 ratio is higher in the RV. The implication is that the RV may be over-perfused or the LV may be under-perfused. The data suggest that the RC resistance, although higher than that observed in the LC vasculature, is not high enough to result in a RV O_2E similar to the LV. Clearly, resting left and right coronary resistances are regulated differently. Further research is required to account for the difference.

In addition, the implications of the results of this research are that the large RV extraction and flow reserves protect the RV myocardium against acute decreases in the CBF/MVO_2 ratio. Clinically, pulmonary hypertension would result in a decrease in the RV O_2E reserve. During further increases in RV oxygen demand, the increase in RCBF would contribute to a greater extent to the increase in oxygen supply. Similar observations would result during RV hypertrophy associated with a chronically elevated pulmonary afterload.

In the first manuscript, RCBF was not affected during the treatments, with the exception of the isoproterenol intervention. RCBF increased dramatically during this condition. It was also noted that during the exercise protocol described within the second manuscript, a similar flow response was observed at the highest level of exercise. It appears that the RV O_2E reserve was exhausted, necessitating an increase in RCBF. It is possible that the combined effects of increased sympathetic nervous activity and increased circulating catecholamines

significantly affected RC resistance during the highest level of exercise intensity. If true, this may account for the increase in RCBF at this level, due to direct β_2-mediated vasodilation. It should be noted, that the effects of isoproterenol cannot definitively be assigned to β_2-mediated vasodilation. Further studies, utilizing a selective β_2-blocker during isoproterenol infusion, will be required to make this conclusion.

CHAPTER VI

PROPOSAL FOR FUTURE RESEARCH

In the vast majority of research, data collected by investigators result in more questions than answers. This investigation is no different. While our study resulted in important new data describing the mechanisms of oxygen supply/demand balance, further investigations are required. Initially, the control of right coronary vascular tone must be determined. Use of the pharmacologic blockade of various vasoactive pathways (i.e. K^+_{ATP} channel blockers, adenosine receptor blockers, or nitric oxide synthase inhibitors) could provide insight into the mechanisms of matching myocardial oxygen supply with demand. Similar techniques could be utilized to examine these issues during dynamic exercise.

In the first manuscript, it was suggested that the direct β_2-mediated vasodilation was responsible for the large increase in RCBF during the infusion of isoproterenol. In order to substantiate this claim, this intervention should be again performed, with the addition of a β_2-specific blocker. The increase in RV

MVO_2 would still be obtained, and the direct vasodilatory properties would be removed.

Our model provides an excellent method for examining the effects of right coronary artery stenosis, pulmonary hypertension (acute and chronic), pulmonary artery constriction, or volume overload on right ventricular oxygen supply and demand. The uses of RCA occluders, pulmonary artery inflation catheters, ameroid constrictors, or pulmonary banding in newborn animals are all possibilities for future experiments. The data collected during these investigations would be of interest to clinicians and clinical investigators.

The differences that exist between the left and right ventricles, as described in our investigation, may be due to different mediators of tissue oxygen demand and oxygen supply. Further investigation is required to determine the mechanisms of the observed differences.

INTRODUCTION TO APPENDIX

Within the two previous investigations, conclusions are derived from the analyses of collected data. It is, therefore, imperative that the data collected is accurate and reliable. Perhaps the two most important data (flow and oxygen extraction) were dependent on the blood samples collected and the direct measurement of coronary blood flow. To evaluate the accuracy of these measurements, possible sources of variation were evaluated, and these are presented in the Appendix. In addition, it is acknowledged that the LV blood flow data utilized for ventricular comparisons in the second manuscript was noticeably lower than that reported in several other LV investigations. Therefore, LV data collected in other investigations was included in the figure for comparison, and are presented in this appendix. Data are also presented that were collected during RV hypoperfusion and were not included within either manuscript.

APPENDIX

Section I

VALIDATION OF PROCEDURES TO ASSESS RIGHT CORONARY OXYGEN CONTENT AND CONSUMPTION

Since the pO_2 values of right coronary blood samples taken with our technique were surprisingly high compared to those found in left ventricular venous samples, the results raise the concern that the venous samples may be contaminated with blood having a higher oxygen content from the right atrium. It was, therefore, imperative to conduct an investigation concerning this issue.

To investigate whether there was contamination from right atrial blood being withdrawn in a retrograde direction, radioactive microspheres were infused simultaneously into the superior and inferior vena cavae. During this time, blood samples were collected from the right atrium and the right coronary vein and later analyzed for radioactivity. Since the microspheres were trapped within the pulmonary circulation, any radioactivity within the coronary venous samples would have come from right atrial contamination. The blood samples did not contain any significant radioactivity (see Table 1 below). The results from this study demonstrate that there was no right atrial blood withdrawn into or mixing

with the right coronary venous sample.

Table 1. Radioactivity counts in right atrial (RA) and right coronary venous (RCV) blood samples.

Time	Dog 1		Dog 2		Dog 3		
(min)	RA	RCV	RA	RCV	RA	RCV1	RCV2
1	626	0	54	5	60	0	0
2	1218	0	7665	0	1424	0	0
3	1182	4	6557	8	1568	8	7
4	1510	0	5881	0	1338	0	0
5	1511	0	7427	5	1516	0	0

It is also possible, given the vascular anatomy of the right heart, that blood from the left ventricular circulation may contribute significantly to right ventricular venous samples through artery-to-artery collaterals, or artery-to-vein and vein-to-vein shunts. For example, a study by Johnston et al. (7) demonstrated that, during complete ligation of the right coronary artery, additional ligation of left ventricular arterial branches resulted in a larger area of RV necrosis. This study suggests that, during complete ligation, collateral vessels contribute to right ventricular metabolism. The current investigation examined right ventricular O_2 handling at rest and during alterations in RV MVO_2. The most critical and difficult measurement was the collection of right coronary venous blood samples.

In the progress of this current investigation, additional data were collected during partial RCA occlusion (see Section IV). The highest potential for venous

sample contamination was during this hypoperfusion protocol, where the RC flow was reduced to 40% of baseline values and the pressure gradient from the left coronary circulation was maximal. This possibility of RV venous contamination with blood from sources other than the right coronary artery was explored in an earlier study by Murakami et al. (8). This group infused Evan's blue dye systemically, while perfusing the RCA from an undyed blood supply. They reported that the left coronary circulation contributed only 4% to right ventricular venous blood samples during RCA hypoperfusion (RCP = 40 mmHg) (8). Using this value, it may be assumed that venous samples collected during this hypoperfusion protocol were potentially composed of 96% RCA blood and 4% of blood from other sources.

To examine whether the left coronary circulation contributed to RV drainage, the multiple sources of RC venous contamination must be considered. As previously mentioned, these sources include artery-to-artery collaterals, artery-to-vein shunts, and vein-to-vein shunts. By assuming 4% contamination from each of these sources independently, the following calculations will explore the amount of error introduced by LV blood. For these calculations, we will utilize values from the literature. The example below is RV MVO_2, as it may be affected by LV blood contamination. The first variable to be included in our calculations is O_2 content. The following values are representative of the literature and our laboratory: Arterial = 18, RV vein = 5, and LV vein = 3 ml O_2/100 ml blood. Then

the following calculations were performed to derive the actual O_2 content of the venous blood, assuming with 4% contamination from the three sources (art.-art., art.-vein, or vein-vein). Values are followed by the % change caused by the contamination.

Oxygen content of the venous sample:

Measured RC Venous O_2 Content = 5.00 ml O_2 / 100 ml blood

A-A = {0.96 * 5} + {0.04 * 5} = 5.00 (0%).

A-V = {0.96 * 5} + {0.04 * 18} = 5.52 (+10%).

V-V = {0.96 * 5} + {0.04 * 3} = 4.92 (-2%).

Therefore, A-A shunt will have no effect, A-V shunt will result in a value 10% higher, and a V-V shunt will cause a 2% lower venous O_2 content measurement. These values are then used to calculate the RV MVO_2 {(A-V $_{\text{Oxygen Content}}$) * RCBF}. RCBF during hypoperfusion (RCP = 40 mmHg) is about 0.20 ml/min/g. Using the above A-V O_2 content difference of 13 (18 - 5 ml O_2/100 ml blood), the RV MVO_2 is 2.6 ml O_2/min/100g. Again, the potential contamination is considered below.

Oxygen consumption of the right ventricle:

Measured RCBF (RCP 40 mmHg) = 0.200 ml/min/g

Measured RV MVO_2 = 20 – 7 {0.200} = 2.6 mlO_2/min/100g

A-A = 18 – 5.00 {0.208} = 2.70 (+4%).

A-V = 18 – 5.52 {0.200} = 2.50 (-4%).

V-V = 18 – 4.92 {0.200} = 2.62 (+1%).

The absolute differences between these calculated values and the "uncontaminated" value (2.6), assuming the 4% contribution from other sources, is 0.1, 0.1, and 0.02 ml O_2/min/100 g for the A-A, A-V, and V-V shunts, respectively. Considering RV MVO_2 calculations of the aforementioned manuscript (8), the standard errors ranged from 0.3 to 0.8, ml O_2/min/100 g, values larger than this contamination error. Therefore, the absolute differences caused by these other sources would not be of detectable significance.

With these calculations, which assume the worst possibilities of RV venous contamination, we are confident that these contamination sources did not significantly affect our calculated values and conclusions.

Section II

FLOWMETER CALIBRATION

The perivascular flowmeter utilized in these investigations was a Transonic® T106 Series model. The flow metering system, patented by Cornell University, uses an ultrasonic transit-time principle to sense liquid volume flow in vessels largely independent of flow velocity, turbulence, or hematocrit. The flowmeter is positioned loosely around the artery, and is insensitive to angular changes. Fibrous encapsulation of the probe occurs within the first week of implant. All Transonic® flowmeters are factory calibrated; however, it was called to our attention that subsequent, extended usage might alter the accuracy. This was a point of concern, as the flowmeters were reused after sterilization.

This matter was addressed using a calibration procedure identical to that utilized by the factory. This technique is diagrammed in Fig 1. The flowmeter was positioned around a thin-walled latex cylinder, representative of a coronary artery. The measured flow was then compared to the actual flow collected into a graduated cylinder. In Fig 2, the time-collected flow is plotted versus the Transonic® flow, for both new, unused probes and the repeatedly used probes.

These relationships are compared with the "perfect" line with slope equal to 1. As indicated, the accuracy of the flow probes after repeated use was not significantly impaired, and the slopes of either plot were not significantly different than 1.

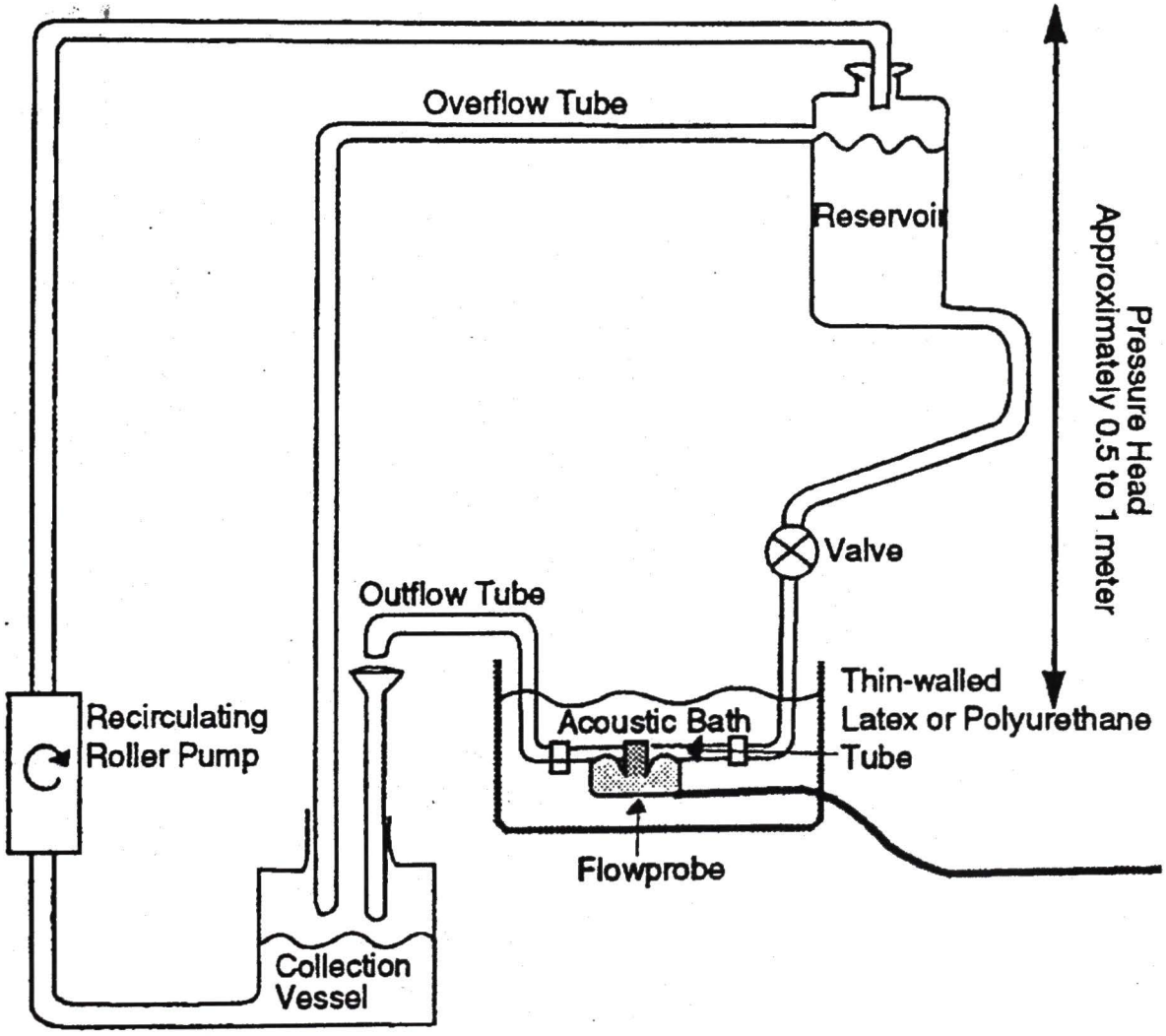


Figure 1. Bench calibration procedure utilized by Transonic® and simulated by our lab for comparison.

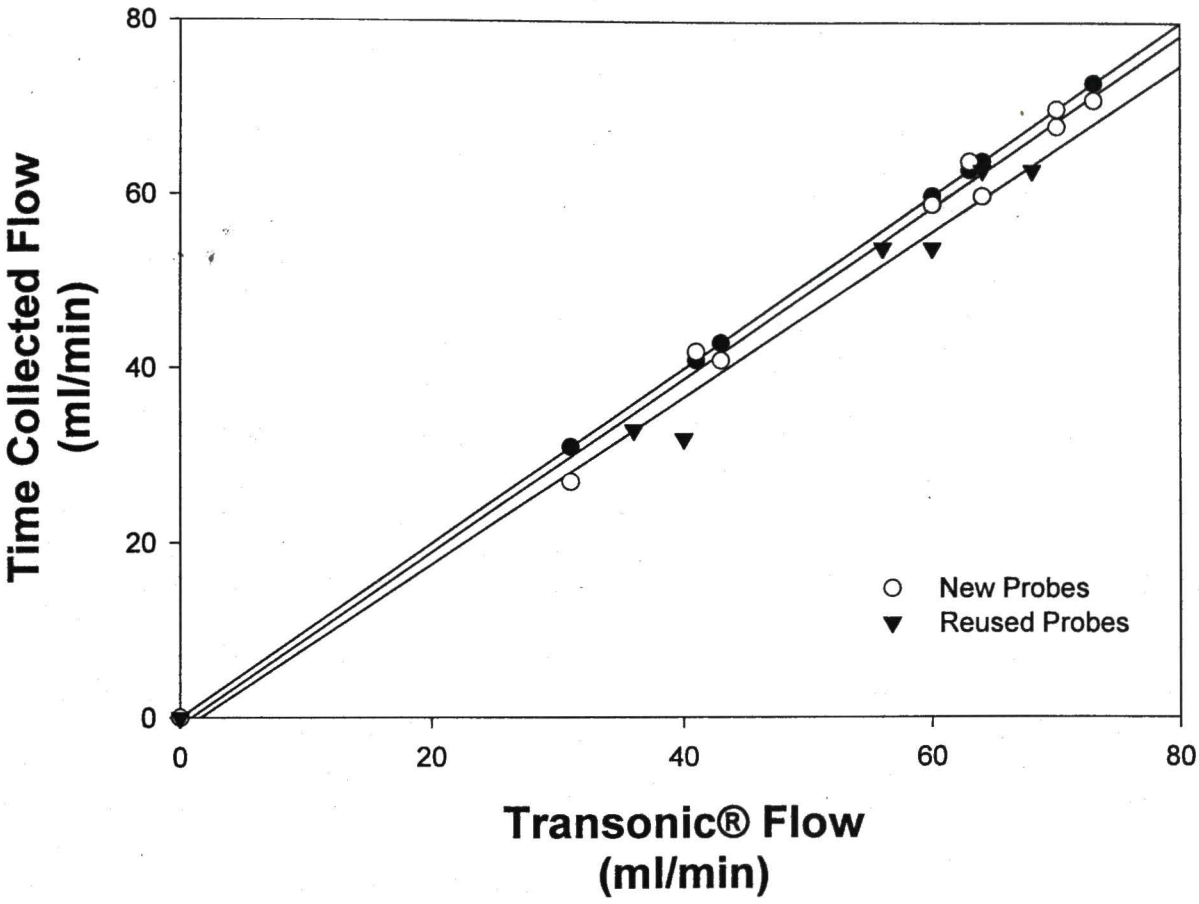


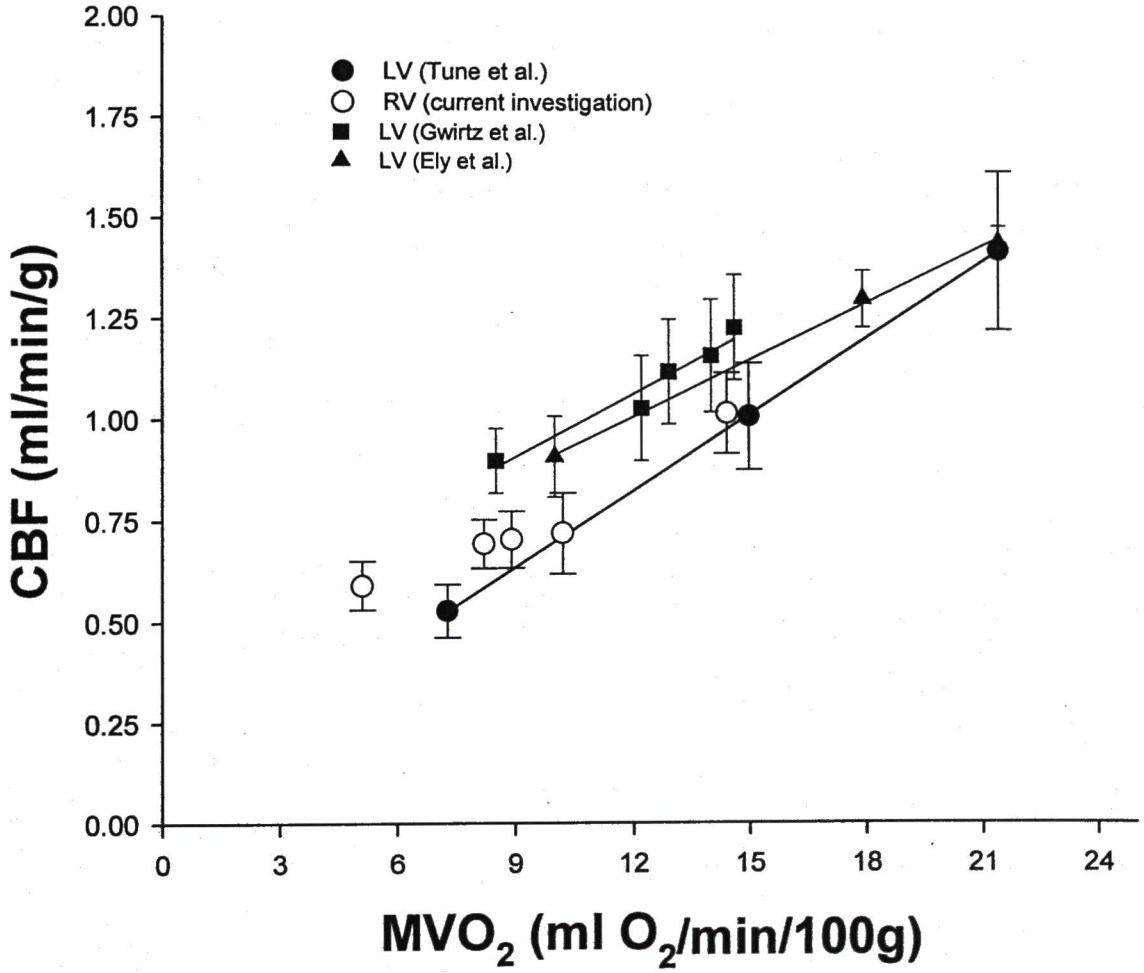
Figure 2. Flowmeter calibration. Closed black circles indicate "perfect" data (Slope = 1.00); open circles, new unused probes (Slope = 0.99); closed triangles, reused probes (Slope = 0.99).

Section III

LEFT VENTRICULAR DATA DURING EXERCISE

In this investigation, data collected during exercise in the RV were compared with data collected during exercise in the LV. The LV data were obtained from a recent publication (10). In this investigation, basal LCBF was substantially lower than other values reported in the literature (2, 3). In Figure 3 that follows, the data reported in these investigations are added to the graph for comparison purposes. Although LCBF values are lower in the Tune investigation than the other two studies, the linear relationship of the variables of CBF and MVO_2 is consistent. Over the range of RV MVO_2 during light to moderate exercise, there is no relationship between RCBF and RV MVO_2. The results of the other investigations indicate a linear relationship in the LV, and support our conclusion that ventricular differences exist in the relationships between oxygen supply variables and oxygen demand.

Figure 3. CBF/MVO_2 Relationship of the Right and Left Ventricles



Section IV

ADDITIONAL DATA COLLECTED DURING RIGHT CORONARY ARTERY HYPOPERFUSION

Acute decreases in myocardial oxygen consumption and contractile function can be induced by reducing right coronary artery perfusion pressure (RCP) (1, 4-6, 8, 9, 11, 12). Reducing RCP from 100 to 60, 40, and 30 mmHg has been extensively utilized to create a condition of hypoperfusion in anesthetized dogs, and these studies have consistently reported a reduced RV MVO_2 that parallels the decrease in CBF (1, 4-6, 8, 9, 11, 12). Under these conditions, the O_2E invariably increases in an attempt to compensate for the decrease in blood supply.

No data exist in the literature describing RV oxygen supply/demand balance in the conscious animal model. An alternate specific aim of this investigation was to collect preliminary data concerning this matter. This aim was to determine the relative alterations of oxygen extraction and contractile function (%SS) during acute decreases in RV oxygen supply. The decrease in supply was created by partial occlusion of the RCA (RCBF = 40% of baseline flow). We hypothesized that during partial coronary artery occlusion, RV function would greatly decrease to limit RV MVO_2 while maintaining an extraction reserve.

The experiment demonstrated that a 60% reduction in flow results in an increase in RV O_2E and a 40% reduction in RV %SS (see Table 2). We concluded that, since this O_2E value increased to a greater extent during the exercise protocol, there remains a potential oxygen extraction reserve during RCA hypoperfusion while regional function is limited.

Table 2. Hemodynamic data during hypoperfusion.

	AoP (mmHg)	HR (beats/min)	RVP_{max} (mmHg)	SS (%)	PvO_2 (mmHg)	O_2 Extr (%)	RCBF (ml/min/g)	MVO_2 (ml O_2/ min/100 g)
Hypoperfusion (n=6)	96±6 (99±4)	105±5 (99±4)	16±2* (19±1)	5.3±1.4* (9.0±1.4)	21±2* (33±1)	61±3* (46±2)	0.21±0.02* (0.51±0.02)	3.01±0.11* (4.70±0.09)

Values are means ± SEM. Values in parentheses are respective baseline values. AoP, mean aortic pressure; HR, heart rate; RVP_{max}, right ventricular peak systolic pressure; SS, % segmental shortening; PvO_2, right coronary venous pO_2; O2 Extr, oxygen extraction; GU, glucose uptake; LU, lactate uptake. * $p<0.05$ vs. untreated respective baseline.

Section V

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