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**PARTIAL vs. FULL SUPPORT OF THE HEART WITH A CONTINUOUS-FLOW
LEFT VENTRICULAR ASSIST DEVICE: IMPLICATIONS FOR
MYOCARDIAL RECOVERY**

by

Carlo Renato G Bartoli
B.S. Cornell University, 2004
M.L.A. Harvard University, 2007
M.S. University of Louisville, 2010

A Dissertation
Submitted to the Faculty of the
Graduate School of the University of Louisville
In Partial Fulfillment of the Requirements
For the Degree of

Doctor of Philosophy

Department of Physiology and Biophysics
University of Louisville
Louisville, KY

May 2011

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A Dissertation Approved on

April 14, 2011

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DEDICATION

This dissertation is dedicated to my father, Dr. Renato Bartoli, and my mother, Anna Bartoli, who have made countless sacrifices so that I may pursue my dream to become a cardiac surgeon and physician scientist. Thank you for teaching me the power of education, hard work, and self-discipline.

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ABSTRACT

PARTIAL vs. FULL SUPPORT OF THE HEART WITH A CONTINUOUS-FLOW LEFT VENTRICULAR ASSIST DEVICE: IMPLICATIONS FOR MYOCARDIAL RECOVERY

Carlo Renato G Bartoli

April 14, 2011

INTRODUCTION: Heart failure is a major and growing public health concern. Although heart failure has been considered an inexorable and progressive disorder, emerging evidence suggests that some patients may have reversible left ventricular dysfunction. Indeed, recent reports have documented the potential for myocardial recovery in humans in response to prolonged mechanical circulatory support with a left ventricular assist device (LVAD). However, myocardial recovery remains uncommon, and a strategy of unloading the failing left ventricle with a continuous-flow (non-pulsatile) LVAD has not been specifically developed to promote favorable myocardial remodeling. As a preliminary investigation, we developed a bovine model of chronic, ischemic heart failure and quantified the effects of different levels of support with a continuous-flow LVAD on myocardial mechanoenergetics.

METHODS: Normal cows (n=8) and cows with chronic, ischemic heart failure (n=9) were studied. To induce heart failure, 90 μ m microspheres were percutaneously injected into the

left main coronary artery. Heart failure developed over 60 days. In an acute surgery, a continuous-flow LVAD was implanted and operated at Low Partial Support (~1.5 L/min support, aortic valve opening every beat), High Partial Support (~3 L/min support, aortic valve opening every beat) and Full Support (~5 L/min, aortic valve maintained closed, left ventricle maximally unloaded). Cardiac and systemic arterial hemodynamics were measured with flow probes and pressure catheters. Myocardial blood flow was mapped with 15 μ m fluorescent-labeled microspheres. After termination, molecular and histological markers of heart failure were quantified.

RESULTS: In normal animals, increasing levels of non-pulsatile support deranged the profile of cardiac and arterial hemodynamics. As cardiac workload decreased, myocardial vascular resistance increased, and myocardial blood flow decreased. The ratio between blood supply and demand did not change and indicated appropriate coronary autoregulation and the presence of an intact coronary reserve. Animals with chronic, ischemic heart failure exhibited hallmark signs of severe left ventricular systolic dysfunction that included a 50% reduction in ejection fraction, left ventricular dilatation, decreased cardiac output and arterial pressures, decreased end-organ blood flow, severe myocardial fibrosis, myocyte hypertrophy, and increased myocardial apoptosis. In animals with chronic heart failure, increasing levels of non-pulsatile support similarly deranged the profile of cardiac and arterial hemodynamics. As cardiac workload decreased, myocardial vascular resistance increased. However myocardial blood flow did not change and indicated a lack of a coronary reserve. Importantly, during full but not partial support, the ratio between blood supply and demand improved significantly to levels observed in normal control animals.

CONCLUSIONS: After the implantation of an LVAD, full but not partial support of the failing left ventricle with an LVAD normalizes the myocardial blood supply/demand relationship. In the immediate postoperative period, the left ventricle should be completely unloaded. Chronic studies are necessary to determine whether a transition to partial support may prevent myocardial atrophy and fibrosis that is seen with prolonged full support. Our bovine model of chronic, ischemic heart failure is appropriate for such a study.

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CHAPTER I

INTRODUCTION

Adult patients with advanced heart failure that is refractory to pharmacological and electrical resynchronization therapies have a limited prognosis. For select patients, heart transplantation offers the best opportunity for long-term survival. However, the number of available donor hearts (~3,500 annually worldwide) are inadequate to meet the needs of more than 30,000 patients listed for heart transplantation worldwide each year¹. As a result, cardiac transplant waiting lists have the highest mortality rate (30%) of any of the solid organ waiting lists².

If a patient decompensates while on a transplant waiting list and he or she is not granted high-urgency status, or if the rate of decompensation is rapid, limited options exist. If a patient does receive a donor heart, chronic rejection and sequelae of long-term immunosuppression limit post-transplant survival to 55% at 10 years³. Furthermore, many patients are not considered for transplantation due to age, comorbidities, or even inadequate insurance coverage. As a result, many patients are being considered for therapy with a permanent, left ventricular assist device (LVAD). Indeed, over the past decade mechanical circulatory support therapies have emerged as a standard, long-term

therapy for adult patients with advanced, intractable heart failure as a bridge to cardiac transplantation⁴, a destination therapy⁴, or a bridge to myocardial recovery⁵⁻⁹.

The recent, widespread success of mechanical circulatory support has ushered in a new era of cardiovascular medicine in which multiple implantable options exist to treat advanced heart failure. Currently, more than 20 novel cardiac assist devices are under development or in clinical trials with nearly a dozen new systems poised to begin clinical trials during the first half of the decade¹⁰⁻²⁵. Each device entails unique surgical and physiological considerations and offers benefits and drawbacks for the patient and the physician. For example, currently available LVADs require extensive, invasive surgery such as sternotomy or thoracotomy. However, these “full-support” devices are able to salvage end-stage heart failure patients by replacing cardiac function and reinstating an adequate circulation. Consequently, full-support devices are reserved as a final treatment option only for patients with life-threatening heart failure. As such, full-support LVADs have not been specifically designed to recover a sick heart, and physicians may be reluctant to refer less-sick patients for an invasive therapy.

In order to expand the role of mechanical circulatory support for the treatment of less-severe stages of heart failure, investigators and industry partners are miniaturizing LVADs for less-invasive and earlier therapy. Small devices that are designed to provide moderate or “partial-support” in heart failure patients with less advanced disease may be employed before the onset of irreversible myocardial damage and end-organ dysfunction. It has been speculated that partial unloading of the failing left ventricle will interrupt the

progressive hemodynamic deterioration observed in heart failure¹⁴ as well as increase functional capacity and improve quality of life. Partial support initiated in patients with less severe myocardial damage may increase the rate of myocardial recovery. Although there is limited data to support these hypotheses, initial clinical results with partial-support devices are encouraging^{14, 24, 26, 27}.

As mechanical circulatory support gains prevalence in the clinical management of cardiovascular disease, it is increasingly important to characterize the physiological response to different devices and mechanical strategies to support the failing heart. Each new LVAD system unloads the left ventricle by a distinctive mechanism. Pulsatile devices mimic pulsatile heart function, whereas continuous-flow devices continuously (and non-physiologically) unload the heart. Full-support devices replace intrinsic cardiac function, whereas partial-support devices augment native hemodynamics. The diversity of strategies to support the failing circulation, the anticipated arrival of numerous novel devices, and the increasing prevalence of patients undergoing long-term mechanical circulatory support raise important basic physiological and clinical questions.

For example, what is the optimal strategy of support to promote myocardial recovery with an LVAD? Mounting evidence suggests that full support of the failing left ventricle with an LVAD can reverse pathologic myocardial remodeling and permit LVAD explantation in select patients⁵⁻⁹. Yet, strategies to promote myocardial recovery with a full-support LVAD have demonstrated limited success. In fact, the current strategy of prolonged full support of patients in end-stage heart failure with irreversible myocardial

damage may actually be detrimental to the cardiovascular system. As the level of continuous unloading increases, the native workload of the heart decreases²⁸. During full support, cardiac workload is nearly eliminated because native cardiac function is no longer needed for adequate circulation. As a result, myocyte atrophy²⁹⁻³¹ and ventricular stiffening^{31, 32} may occur and preclude myocardial recovery. Therefore, an understanding of effects of mechanical unloading of the heart on myocardial mechanoenergetics is needed in order to develop better support strategies to promote myocardial recovery.

It is generally recognized that varying the level of support with an LVAD affects myocardial workload and coronary blood flow³³. Yet this relationship has not been well characterized and the implications of altered workload and coronary blood flow for myocardial recovery are unclear. As a starting point, characterization of the blood supply/demand relationship during LVAD support may answer fundamental questions necessary to begin to develop a protocol to promote myocardial recovery with a continuous-flow LVAD. The purpose of this dissertation project was to examine cardiac and arterial hemodynamics, coronary blood flow, and myocardial workload during varying levels of support with a continuous-flow LVAD²⁸. To this end, a bovine model of chronic heart failure was developed. Continuous-flow LVADs were implanted in normal calves and calves with chronic, ischemic heart failure. During partial and full support with a continuous-flow LVAD, hemodynamics and myocardial blood flow were measured. The data presented within this dissertation document four important findings:

- 1) **Left main coronary artery microembolization induced chronic, ischemic heart failure in calves. A stable and reproducible large-animal model of chronic heart failure is possible with many phenotypic similarities to clinical heart failure.**
- 2) **In normal animals and animals with chronic heart failure, full but not partial support with a continuous-flow LVAD deranged the physiological profile of pulsatile cardiac and arterial hemodynamics.**
- 3) **In normal animals, neither full nor partial support with a continuous-flow LVAD affected the myocardial blood supply/demand relationship. However, in animals with chronic heart failure, full but not partial support normalized the myocardial blood supply/demand relationship.**
- 4) **Divergent results were observed between normal animals and animals with chronic heart failure. Normal animals do not reproduce the complex pathophysiological presentation of chronic heart failure and are not ideal for validation and proper translation of mechanical circulatory support strategies into clinical practice.**

The major finding of this study is that in the immediate postoperative period after the implantation of an LVAD, complete unloading of the failing left ventricle will rebalance the myocardial blood supply/demand relationship. However, full support with a

continuous-flow LVAD dramatically changes cardiac and systemic arterial hemodynamics and may have long-term consequences. Therefore, chronic studies are necessary to determine whether a transition to partial support may alleviate or prevent myocardial atrophy and fibrosis that is seen with prolonged full support. Our bovine model of chronic, ischemic heart failure is appropriate for such a study.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

I. Pulsatility during Mechanical Circulatory Support

Over the past two decades, full-support LVADs have evolved into a standard therapy for patients with end-stage heart failure. In 2002, the milestone Randomized Evaluation of Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH) trial demonstrated clinical success with pulsatile LVADs as a long-term therapy for end-stage heart failure patients³⁴. Subsequently, the United States Food and Drug Administration (FDA) approved use of the first-generation, pulsatile HeartMate XVE (**Figure 1, Left**) as a destination therapy in patients ineligible for cardiac transplantation³⁵.

More recently, pulsatile LVADs, which mimic pulsatile cardiac function, have been replaced by rotary blood pumps, which continuously (and non-physiologically) unload the left ventricle. In 2008, the FDA approved use of the HeartMate II (**Figure 1, Right**) continuous-flow LVAD in patients for bridge-to-transplant therapy³⁶. Such next-generation, full-support rotary pumps are smaller, more reliable, more energy efficient, and less surgically traumatic to implant. Notably, superior clinical outcomes have been

established with next-generation continuous-flow devices³⁷. Yet continuous-flow LVADs do not generate normal pulsatility (**Figure 2**)²⁸. As a result, a new population of “pulseless” patients undergoing long-term LVAD support has emerged.

Figure 1, Left: A pulsatile HeartMate XVE is shown. First generation pulsatile devices mimic native cardiac function. However, these devices are large, difficult to implant, and less reliable for long-term therapy. **Right:** A continuous-flow (non-pulsatile) HeartMate II is shown. Second generation continuous-flow devices are smaller, easier to implant, and operate with excellent durability for prolonged LVAD therapy. However, the non-pulsatile mechanism dramatically alters the circulation of blood.

Figure 2, Left: Full support with a pulsatile LVAD demonstrates a normal pulsatile arterial pressure. As blood volume is removed from the left ventricle in a phasic manner, an arterial pulse is generated. Additionally, sufficient preload permits the left ventricle to eject through the aortic valve, which preserves a normal pulsatile circulation. **Right:** In contrast, full support with a continuous-flow LVAD demonstrates a nearly flat arterial pressure. As blood volume is continuously removed from the left ventricle into the aorta, insufficient preload prevents ejection of blood through the aortic valve. As a result, left ventricular pressure never exceeds aortic pressure, the aortic valve remains chronically closed, and the systemic circulation is non-pulsatile. AoP, aortic pressure; LVP, left ventricular pressure

In this patient population, investigators have raised concerns about the effect of chronic, non-pulsatile blood flow on myocardial and end-organ perfusion, cardiovascular architecture, and long-term patient outcomes³⁸.

Pulsatility and mechanical stretch of cardiac myocytes, endothelial cells, and vascular smooth muscle cells are fundamental to cardiovascular homeostasis. On day 26 of human embryogenesis, the immature fetal heart begins to beat and pump blood through a rudimentary vascular system³⁹. From this day forward until the last day of life, cardiac contraction, stroke volume ejection, and cardiac relaxation with one-way valves expose every cardiovascular cell (and most cells in the body) to rhythmic mechanical forces that mediate intracellular and extracellular events. Seemingly, pulsatility is a vital component of normal mammalian physiology.

Indeed, numerous investigators have demonstrated the superiority of pulsatile blood flow over non-pulsatile flow during short-term and long-term mechanical circulatory support in experimental models as well as in adult and pediatric patients⁴⁰⁻⁵². However, despite the evidence in favor of pulsatile perfusion techniques, a debate still exists as to which mode of circulatory support is better. A separate set of investigators have reported no difference between continuous and pulsatile perfusion modes⁵³⁻⁵⁹.

Nevertheless, continuous-flow LVADs constitute the current clinical gold standard for prolonged mechanical circulatory support³⁷. Yet the effect of continuous support of the left ventricle and reduced pulsatility on cardiac workload, coronary blood flow, and

regional myocardial perfusion remain largely uncharacterized. During heart failure, the supply/demand ratio of available oxygen in blood to myocyte workload becomes unbalanced⁶⁰. Likely, appropriate support with an LVAD may rebalance this relationship, which may have important implications as the initial step toward myocardial recovery.

II. Partial vs. Full Mechanical Unloading of the Failing Left Ventricle

If a patient awaiting a cardiac transplant decompensates and an appropriate organ is not available, a full-support LVAD may be the only option for survival. Yet many heart failure patients are not ill enough or have contraindications to placement of a full-support LVAD. In these patients, the notion of combining the benefits of partial support with a minimally invasive and short operation without cardiopulmonary bypass may be feasible.

A recent National Heart, Lung, and Blood Institute (NHLBI) mission statement included the pursuit of long-term hemodynamic support with minimally invasive surgery to provide moderate levels of mechanical assistance earlier in the progression of heart failure (NHLBI, Clinical Use of Ventricular Assist Devices Working Group, March 27-28, 2008 Crystal City, VA)⁶¹. The long-term benefits of chronic, partial support are unknown but may soon be partially characterized by ongoing clinical trials¹⁴. Significant clinical benefits have been predicted⁶² that may decrease the number of patients that require a heart transplant.

For example, a large gap in available therapies exists for patients in NYHA class III heart failure that have not responded to biventricular pacing. If these patients are transplant ineligible or have not met hemodynamic and clinical criteria to justify risks and comorbidities of sternotomy and a full-support LVAD, limited options exist. In these patients, partial support with a less-invasive device is an attractive option. If partial support is administered early enough, favorable reverse myocardial remodeling may

restore sufficient native cardiac function and permit explantation of the device⁷. This hypothesis has not been rigorously tested but is conceptually appealing.

Indeed, in rare instances, full support with an LVAD has resulted in myocardial recovery⁵⁻⁹. In these patients, functional recovery has accompanied favorable changes at the molecular and histological levels^{7, 63, 64}. Yet, strategies to promote reverse myocardial remodeling with a full-support LVAD have demonstrated limited success and myocardial recovery remains uncommon. As it turns out, prolonged full support of the left ventricle with a continuous-flow LVAD may produce numerous unfavorable results. For example, as the level of full-support increases, variation in end-systolic and end-diastolic volumes diminish and eliminate the native workload of the heart²⁸. Myocyte atrophy^{29, 30} and ventricular stiffening³² may occur and produce a noncompliant ventricular chamber incapable of sustained, independent function. Simultaneously, complete volume unloading of the left ventricle decreases peak systolic pressures to the point where the aortic valve remains chronically closed. As a result, fused valve leaflets, acquired aortic stenosis, or total occlusive thrombosis of prosthetic aortic valves may occur⁶⁵. Excessive ventricular unloading with an LVAD may also result in suction events and ventricular collapse, which may trigger episodes of fatal ventricular tachyarrhythmias^{66, 67}.

Of additional concern, during therapy with a full-support LVAD, approximately 30% of patients develop right ventricular dysfunction⁶⁸ with an associated mortality rate of 43%⁶⁹. During left heart failure, full support with an LVAD may abruptly increase systemic arterial flow, acutely overload the right ventricle, and cause right ventricular

failure. For these reasons, full support of the left ventricle with a continuous-flow LVAD may cause adverse consequences and limit myocardial recovery.

Furthermore, patients in NYHA class III or early class IV heart failure with less-advanced disease may not require full support with an LVAD. Accordingly, rather than completely unloading the failing heart, earlier and partial unloading may reduce but not eliminate native ventricular workload, preserve myocardial structure, and prevent myocyte atrophy and ventricular stiffening. By reducing ventricular workload and augmenting myocardial blood flow while still allowing the heart to fill and empty over a controlled range of ventricular volumes, partial support may be an effective strategy not only to augment hemodynamics but also to promote favorable myocardial remodeling in hearts with less disease. Importantly, patients with healthier hearts may have a higher likelihood of myocardial recovery⁶².

With this strategy in mind, preclinical studies are necessary to determine effects of partial support on cardiovascular physiology. Therefore, the purpose of this dissertation project was to initiate *in vivo*, large-animal experiments to characterize the effect of full and partial support with a continuous-flow LVAD on systemic hemodynamics and the myocardial blood supply/demand relationship. Our findings have important clinical utility and may increase the public-health impact of mechanical circulatory support with next-generation, continuous-flow LVADs.

CHAPTER III

FUNDAMENTAL QUESTIONS

I. Phenomenon

Full-support devices constitute the current gold standard for the treatment of end-stage heart failure with an LVAD. Myocardial recovery with a continuous-flow LVAD is rare, and effective strategies to facilitate reverse myocardial remodeling have not been developed. The field of mechanical circulatory support is trending toward miniaturized LVADs that can be implanted with minimally invasive surgery and that provide partial support for earlier therapy in healthier patients. The clinical utility of partial support is unknown. However, partial support of patients in earlier stages of heart failure may be a better strategy to promote myocardial recovery.

II. Proposed Concepts

Important hemodynamic differences exist between partial vs. full support of the cardiovascular system. The effect of continuous unloading of the left ventricle on cardiac and systemic hemodynamics, myocardial workload, and coronary blood flow is not well characterized. The effect of partial support with a continuous-flow LVAD on myocardial

mechanoenergetics is unknown, and the clinical role of partial support is yet to be defined.

III. *Experimental Design*

A series of experiments were designed to compare effects of partial vs. full support with a continuous-flow LVAD on cardiac and systemic hemodynamics, myocardial workload, and coronary blood flow. In normal animals without cardiovascular pathology, continuous-flow LVADs were implanted to determine effects of partial support in the normal cardiovascular system. In parallel, a model of chronic, ischemic heart failure was developed in calves. Experiments were repeated in the heart failure model to determine effects of partial support in the failing cardiovascular system.

IV. *Hypotheses and Specific Aims*

Hypothesis #1: In normal animals, increasing levels of support with a continuous-flow LVAD will alter the pulsatile cardiac and systemic arterial hemodynamic profile.

Continuous mechanical unloading of the *normal* left ventricle will reduce left ventricular but not right ventricular workload and blood flow in a manner dependent on the degree of support. The left ventricular blood supply/demand relationship will not change.

Aim #1: Characterize and Compare Hemodynamics and Myocardial Blood Flow during Partial and Full Unloading of the *Normal* Left Ventricle with a Continuous-Flow LVAD

The goals of this aim were to determine how support with a continuous-flow LVAD affected hemodynamics, cardiac workload, and myocardial blood flow in normal animals. Regional myocardial blood flow was related to cardiac and arterial hemodynamics.

Hypothesis #2: Coronary microembolization will produce stable and reproducible chronic, ischemic heart failure in calves.

Aim #2: Develop and Characterize a Bovine Model of Chronic Heart Failure

The goals of this aim were to establish and characterize a stable and reproducible model of microembolization-induced chronic, ischemic heart failure. This model was used to test the hypothesis of Aim #3.

Hypothesis #3: In animals with chronic heart failure, increasing levels of support with a continuous-flow LVAD will alter the pulsatile cardiac and systemic arterial hemodynamic profile. Continuous mechanical unloading of the *failing* left ventricle will reduce left ventricular but not right ventricular workload in a manner dependent on the degree of support. As a result, the unbalanced blood supply/demand relationship in the chronically hypoperfused myocardium will rebalance.

Aim #3: Characterize and Compare Hemodynamics and Myocardial Blood Flow during Partial and Full Unloading of the *Failing* Left Ventricle with a Continuous-Flow LVAD

The goals of this aim were to determine how support with a continuous-flow LVAD affected hemodynamics, cardiac workload, and myocardial blood flow in animals with chronic heart failure. Regional myocardial blood flow was related to cardiac and arterial hemodynamics.

CHAPTER IV

Aim #1: PARTIAL vs. FULL SUPPORT OF THE NORMAL LEFT VENTRICLE WITH A CONTINUOUS-FLOW LVAD

I. Introduction

Ongoing theoretical debate exists regarding the merits and limitations of partial vs. full circulatory support. With nearly 3,000 patients logged, the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) recently documented a 1-year survival rate of 75% with full-support LVADs⁷⁰. Clearly, end-stage heart failure patients are benefiting from full-support LVADs. Yet, better clinical outcomes are necessary before prolonged LVAD support is more widely accepted. For example, currently approved devices contain parts that wear down such as polymeric valves and diaphragms or mechanical contact bearings. To counter this limitation and increase device durability, third-generation, full-support devices include a bearingless, magnetically suspended impeller that does not wear over time or generate frictional heat¹³. As a result, many continuous-flow devices have been miniaturized. In patients

with moderate heart failure, earlier and minimally invasive partial support may preserve a near-normal pulsatile hemodynamic profile and reduce but not eliminate cardiac workload.

Partial support with a minimally invasive surgical approach may also expand the potential patient population for LVAD therapy. As LVADs are miniaturized, less-invasive surgical approaches may increase acceptance by physicians who are more likely to refer patients for minimally invasive therapies⁷¹. Earlier intervention in less-sick patients should increase the public-health impact of mechanical circulatory support.

In our laboratory, preliminary work in a mock circulatory system demonstrated marked differences in pulsatility, ventricular pressure-volume relationships, and coronary blood flow during partial vs. full support with a continuous-flow LVAD⁷². However, in this *in vitro* study it was not possible to determine the effect of partial support on regional myocardial blood flow or the myocardial blood supply/demand relationship. A recent study in sheep demonstrated that increasing levels of support with a continuous-flow LVAD progressively decreased myocardial oxygen consumption, and coronary blood flow did not change³³. However, the authors did not normalize coronary blood flow to myocardial oxygen consumption, and the effect on the oxygen supply/demand ratio was not reported. Furthermore, this study was performed in normal animals without cardiovascular pathology.

In order to more rigorously characterize the effect of continuous unloading on the myocardial blood supply/demand relationship, Aim #1 was a first step in understanding the chronic consequences of full vs. partial support with a continuous-flow LVAD. The major goal was to examine acute responses in normal animals. Standard indices of cardiovascular performance and regional myocardial blood flow were measured during partial and full support of the normal left ventricle. The implication for reverse myocardial remodeling and myocardial recovery were considered. The results of this study will help to guide future investigation into acute and chronic effects of continuous-flow mechanical circulatory support in large-animal models of chronic cardiac pathology.

II. *Materials and Methods*

Animals

Male Jersey and mixed-breed calves (n=8, 126±13 kg) were used. All animals received humane care and were handled in accordance with National Institutes of Health and University of Louisville animal care committee guidelines. Experimental procedures followed the University of Louisville Institutional Animal Care and Usage Committee approved protocol #08073.

Anesthesia

Animals were pre-anesthetized with Atropine (30 mg) and prepared for acute, non-sterile surgery. In the operating room, general anesthesia was administered with Isoflurane (3-5%) and room air. The animal was placed on the operating table in right lateral recumbency. Tidal volume and respiratory rate were adjusted to maintain arterial oxygen saturation above 90%. Fluid-filled arterial and venous catheters were placed in the left carotid artery and jugular vein for blood sampling. A left thoracotomy was performed. Ribs #4 and #5 were resected. The pericardium was opened. The animal was anticoagulated with a single bolus of intravenous Heparin (100 units/kg). For the remainder of the procedure, the activated clotting time (ACT) was maintained above 250 s with additional boluses of Heparin (1,000 to 2,000 units).

Surgical Instrumentation

A single-tip high-fidelity micromanometer catheter (Millar Instruments, Houston, TX)

was placed in the aorta and a dual pressure-volume conductance catheter (Millar Instruments, Houston, TX) was advanced from the left atrium across the mitral valve into the left ventricle for simultaneous measurement of aortic, left atrial, and left ventricular blood pressures. A transit-time ultrasonic flow probe (Transonics, Ithaca, NY) was placed around the pulmonary artery to measure cardiac output. In six animals, a silicone catheter (7-French, Access Technologies, Skokie, IL) was advanced in the left atrial appendage chamber for administration of 15 μ m fluorescent-labeled polystyrene microspheres as described below. The depth and angle of catheter entry parallel to the surface of the left atrial appendage ensured that the catheter did not interfere with mitral valve function.

LVAD Implantation

A continuous-flow LVAD (HeartWare HVAD n=4, Thoratec HeartMate II n=2) was implanted with cardiopulmonary bypass. The outflow graft was anastomosed to the descending aorta. The left ventricular apex was cored and cannulated. The device and outflow graft were de-aired. A transit-time ultrasonic flow probe (Transonics, Ithaca, NY) was placed around the outflow graft to measure LVAD flow.

Experimental Design

In each animal, blood pressure and flow waveforms were recorded during Baseline (pump off, outflow graft clamped), Low Partial Support (~1.5 L/min support, aortic valve opening every beat), High Partial Support (~3 L/min support, aortic valve opening every beat), and Full Support (~5 L/min, aortic valve maintained closed, left ventricle

maximally unloaded). Baseline and support modes for each device were maintained for 10 minutes each to achieve steady-state conditions prior to collection of 30 second data sets. During each condition, a single color of fluorescent-labeled microspheres (5.25 million microspheres) was injected into the left atrial catheter. Simultaneously, a reference blood sample was withdrawn from the arterial line at a rate of 15 ml/min for 100 seconds.

Quantification of Microspheres and Regional Myocardial Blood Flow

The microsphere technique enabled the precise measurement of regional blood flow in vascular beds of interest as follows. In the left ventricle, the microspheres mixed with the blood and were ejected into the aorta to disseminate throughout the body to every organ according to the physiological distribution of blood flow during that experimental condition. As the microspheres approached capillaries, they lodged within the smallest pre-capillary arterioles based on regional tissue blood flow patterns. With the quantity used, 15 μm spheres do not cause ischemia and did not induce pathology⁷³.

The aortic blood sample acted as a reference for later determination of flow in tissues of interest. The number of counted microspheres in the reference blood sample (known – counted with flow cytometry) was compared to the number of microspheres that lodged in pre-capillary arterioles and were counted in a tissue sample of interest (known – counted with flow cytometry). The ratio between the two sphere counts was equal to the ratio between the calibrated rate of aortic withdrawal (known – 15 ml/min) and flow in

the tissue of interest (*unknown*) and provided accurate determinations of tissue specific flows in milliliters per minute per gram of tissue (ml/min/g)^{73, 74}.

At the completion of the study, while under anesthesia, euthanasia was performed with a fatal intravenous bolus injection of Beuthanasia-D Special (1 ml/5 kg). The heart was removed. One to two gram tissue sections from the left ventricular free wall, right ventricular free wall, interventricular septum, and left ventricular epicardium, mid-myocardium, and endocardium were collected. Myocardial and reference blood samples were sent to IMT/Stason Laboratories (Irvine, CA) for automated digestion, counting of fluorescent microspheres with flow cytometry, and calculation of tissue specific blood flows in ml/min/g of tissue.

Data Reduction

All transducers were pre- and post-calibrated against known physical standards to ensure measurement accuracy. Calibration curves for the volume conductance catheter were constructed using static and dynamic tests pre- and post-experiment. Data were collected at 400 Hz, signal conditioned, and A/D converted for digital analysis using our Good Laboratory Practices (GLP) compliant data acquisition system⁷⁵.

To determine hemodynamic performance during each support mode, pressure and flow waveforms were used to derive LVAD flow (LVADF), heart rate (HR), left ventricular stroke volume (LV SV), pulmonary artery flow (PAF) as an index of cardiac output (CO), mean left atrial pressure (LAP), left ventricular end-diastolic pressure (LVP_{end}

diastolic), left ventricular peak systolic pressure ($LVP_{\text{peak systolic}}$), $\pm dP/dt$, aortic systolic blood pressure (AoP_{systolic}), aortic diastolic blood pressure ($AoP_{\text{diastolic}}$), mean aortic pressure (AoP_{mean}), and aortic pulse pressure (AoP_{pulse}). Rate-pressure product ($HR \times LVP_{\text{peak systolic}}$), a standard index of cardiac metabolic demand⁷⁶, was calculated.

Myocardial vascular resistance was calculated as $AoP_{\text{mean}}/\text{region-specific myocardial blood flow}$. Hemodynamic variables were calculated on a beat-to-beat basis for each 30 second data set with the Hemodynamic Evaluation and Assessment Research Tool (HEART) program⁷⁷ developed in Matlab (Version 6.5, MathWorks, Natick, MA). All analyzed beats in each data set (approximately 30 to 50 beats/30 second data set) were averaged to obtain a single representative mean value for each calculated variable.

Statistics

GraphPad, version 4.00 (Prism, La Jolla, CA) was used to perform statistical analyses and plot data. One-way repeated measures ANOVA with Tukey post-test was performed for each hemodynamic index, region of myocardial blood flow, and myocardial blood flow normalized to $HR \times LVP_{\text{peak systolic}}$ to compare Baseline, Low Partial Support, High Partial Support, and Full Support modes within each animal. All analyses were two-tailed, and a p-value < 0.05 (95% confidence) was considered statistically significant. All data are presented as mean $\pm$ standard error.

III. Results

Table 1 demonstrated that in normal animals, as the level of support with a continuous-flow LVAD increased, the cardiac and systemic arterial hemodynamic profile progressively changed. An increase in continuous support decreased HR and CO. Although the difference between Baseline and Full Support were not statistically different, the reductions were quantitatively large (HR, -16 bpm; CO, -0.9 L/min).

Left Ventricular Hemodynamics

Left ventricular pressures demonstrated the most robust changes. The progressive increase in continuous support significantly decreased LAP ($p=0.01$), $LVP_{\text{end diastolic}}$ ($p<0.001$), and $LVP_{\text{peak systolic}}$ ($p=0.05$) with a dose-dependent response. These reductions were greatest and indicated maximum unloading during Full Support. Smaller reductions were observed during partial-support modes. Increasing levels of continuous support did not affect $\pm dP/dt$.

During Full Support, the variation between peak-systolic and end-diastolic pressures decreased to non-physiologically low values and resulted in chronic closure of the aortic valve. In this situation, the average $LVP_{\text{peak systolic}}$ (77 ± 11 mmHg) did not exceed the $AoP_{\text{diastolic}}$ (80 ± 6 mmHg). As a result, the aortic valve remained closed, and HR x $LVP_{\text{peak systolic}}$ decreased significantly ($p=0.05$) to values that are not seen in normal animals. This finding indicated that unloading the heart with a continuous-flow LVAD

dramatically reduced myocardial metabolic demands and with a dose-dependent response.

Arterial Hemodynamics

Systemic arterial pressures demonstrated less robust changes. As continuous support increased, AoP_{mean} and $AoP_{systolic}$ did not change. However, during Full Support $AoP_{diastolic}$ trended toward an increase of nearly 10 mmHg ($p=0.11$), and AoP_{pulse} decreased significantly from 28 ± 8 to 14 ± 11 ($p<0.0001$). In contrast, during partial-support modes, the arterial pulse pressure was not significantly changed from baseline.

Myocardial Blood Supply/Demand Relationship

In normal animals, continuous support of the left ventricle did not affect right ventricular myocardial vascular resistance or blood flow. However, in the left ventricle and interventricular septum, increasing levels of continuous support reduced the cardiac workload, increased myocardial vascular resistance (**Table 2**), and decreased regional left ventricular blood flow (**Table 3**) with a significant dose-dependent response. Changes in vascular resistance and blood flow in the epicardial region demonstrated that the coronary arteries and large coronary arterioles were participating in observed changes.

To characterize the relationship between myocardial blood supply/demand, regional myocardial blood flow was normalized to $HR \times LVP_{peak\ systolic}$. Increasing levels of continuous support did not alter the ratio between left ventricular myocardial blood flow and left ventricular workload (**Table 4**). A baseline value of 0.0085

n=8	LVADF (L/min)	HR (bpm)	LV SV (ml)	PAF (L/min)	LAP (mmHg)	LVP _{end diastolic} (mmHg)	LVP _{peak systolic} (mmHg)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)	HR x LVP _{peak systolic} (bpm x mmHg)	AoP _{mean} (mmHg)	AoP _{systolic} (mmHg)	AoP _{diastolic} (mmHg)	AoP _{pulse} (mmHg)
ANOVA p-value	p<0.0001	p=0.08	p=0.61	p=0.24	p=0.01	p<0.001	p=0.05	p=0.68	p=0.34	p=0.05	p=0.93	p=0.68	p=0.11	p<0.0001
Baseline	0.0±0.0	86±7	99±9	8.6±1.1	16±6	15±2	98±5	1,351±204	-1,609±160	8,627±1,085	84±5	100±5	71±5	28±3
Low Partial Support	1.8±0.2*	72±5	108±17	7.9±1.3	15±5	16±2	96±6	1,517±295	-1,698±262	6,935±577	83±5	98±6	69±4	29±3
High Partial Support	3.3±0.3*†	71±5	106±13	7.5±1.1	15±5	15±2	91±8	1,243±241	-1,420±175	6,463±753	83±5	95±5	73±4	22±3†
Full Support	5.2±0.6*†‡	70±5	111±13	7.7±0.9	12±5*	11±2*†‡	77±11	1,296±340	-1,299±349	5,568±971*	86±5	94±6	80±6	14±4*†‡

Table 1: Hemodynamics during partial vs. full unloading of the normal left ventricle with a continuous-flow LVAD. LVADF, left ventricular assist device flow; HR, heart rate; LV SV, left ventricular stroke volume; PAF, pulmonary artery flow; LAP, left atrial pressure; LVP, left ventricular pressure; AoP, aortic pressure; *p<0.05, support mode vs. Baseline; †p<0.05, support mode vs. Low Partial Support; ‡p<0.05, support mode vs. High Partial Support

ml/min/100g/bpm·mmHg was established as the myocardial blood supply/demand relationship in normal animals. **Figure 3** summarizes the gross physiological changes observed during continuous unloading of the normal left ventricle.

n=6	RV	LV	Septum	Epicardium	Mid-Myocardium	Endocardium
	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)
ANOVA p-value	p=0.47	p=0.07	p=0.04	p=0.07	p=0.02	p=0.08
Baseline	138±28	120±22	114±20	138±30	106±20	133±21
Low Partial Support	163±33	151±30	154±28	173±40	138±30	186±41
High Partial Support	235±82	238±86	239±86	262±98	199±65	253±92
Full Support	188±67	284±85	304±86*	328±108	277±75*	352±123

Table 2: Regional myocardial vascular resistance during partial vs. full support of the normal left ventricle with a continuous-flow LVAD. RV, right ventricle; LV, left ventricle; *p<0.05, Full Support vs. Baseline

n=6	RV	LV	Septum	Epicardium	Mid-Myocardium	Endocardium
	(ml/min/g)	(ml/min/g)	(ml/min/g)	(ml/min/g)	(ml/min/g)	(ml/min/g)
ANOVA p-value	p=0.49	p=0.05	p=0.07	p=0.07	p=0.01	p=0.11
Baseline	0.60±0.90	0.70±0.10	0.73±0.13	0.65±0.13	0.80±0.12	0.61±0.08
Low Partial Support	0.47±0.08	0.63±0.14	0.60±0.13	0.58±0.14	0.69±0.14	0.53±0.13
High Partial Support	0.47±0.08	0.52±0.13	0.52±0.14	0.51±0.14	0.58±0.14	0.47±0.12
Full Support	0.49±0.09	0.43±0.11*	0.38±0.09*	0.40±0.10	0.42±0.10*	0.35±0.08

Table 3: Regional myocardial blood flow during partial vs. full support of the normal left ventricle with a continuous-flow LVAD. RV, right ventricle; LV, left ventricle; *p<0.05, Full Support vs. Baseline

n=6	LV/HRxLVP _{peak systolic}	Septum/HRxLVP _{peak systolic}
	(ml/min/100 g/bpm·mmHg)	(ml/min/100 g/bpm·mmHg)
ANOVA p-value	p=0.72	p=0.91
Baseline	0.0082±0.0011	0.0085±0.0014
Low Partial Support	0.0101±0.0017	0.0097±0.0017
High Partial Support	0.0082±0.0017	0.0080±0.0016
Full Support	0.0119±0.0046	0.0093±0.0025

Table 4: Regional myocardial blood supply/demand relationship during partial vs. full support of the normal left ventricle with a continuous-flow LVAD. LV, left ventricle; HR, heart rate; LVP, left ventricular pressure

Figure 3: During support of the normal left ventricle with a continuous-flow LVAD, cardiac workload decreased, myocardial vascular resistance increased, and myocardial blood flow decreased. However, the relationship between blood supply and demand did not change and indicated that an intact coronary reserve and appropriate coronary autoregulation were present.

IV. Conclusions

The study of partial vs. full support of the left ventricle with a continuous-flow LVAD was possible in normal animals. Specifically, in animals without cardiovascular pathology, 1) a systemic flow reserve was present, 2) full support with a continuous-flow LVAD deranged pulsatility whereas partial support preserved a more normal pulsatile profile, 3) as continuous support increased, left ventricular workload and blood flow decreased, likely as a result of appropriate coronary autoregulation and an intact coronary reserve. These results will help guide future acute and chronic studies in large-animal models of cardiac pathology to determine effects of partial vs. full support of the left ventricle with a continuous-flow LVAD.

Systemic Effects

In normal animals, a systemic flow reserve was present. As the level of support with a continuous-flow LVAD increased, HR and CO decreased in a dose-dependent manner. This finding in animals without chronic pathology was not unexpected.

In chronic heart failure, a systemic flow reserve may be absent. Therefore, these results from normal animals likely cannot be generalized to animals with heart failure.

It is possible that a lack of pulsatile blood flow drops systemic metabolism. To generate force, cardiac myocytes consume large quantities of oxygen⁷⁸. Similarly, because of pulsatile stretch and in order to maintain vascular tone, endothelial cells have the greatest oxidative metabolism of any cells in the body⁷⁹. As a result, during the generation and

regulation of arterial pulsatility, cardiac myocytes, endothelial cells, and vascular smooth muscle cells metabolize large quantities of oxygen. Therefore, the reduction of cardiac metabolic demands and arterial pulsatility with a continuous-flow LVAD is likely to drop total body oxygen requirements and therefore CO. If this is the case, any level of support with a continuous-flow LVAD that reduces cardiac workload and arterial pulsatility will reduce total body oxygen consumption and benefit the heart failure condition.

Effects on the Heart

In the normal heart, full support of the left ventricle with a continuous-flow LVAD deranged the natural profile of pulsatile cardiac hemodynamics. As continuous support increased, the maximum pressure generated by the left ventricle dropped below the arterial diastolic pressure. Insufficient preload was available to eject through the aortic valve, which did not open. As a result, the workload of the heart was significantly reduced to values that are not normally observed in bovids. As workload decreased, myocardial vascular resistance increased, and myocardial blood flow decreased. The ratio between blood supply and demand did not change and indicated appropriate coronary autoregulation and the presence of an intact coronary reserve. Substantial changes in epicardial vascular resistance and blood flow further suggested that the coronaries were participating in this process and that coronary vasomotor function was normal.

In chronic heart failure, a coronary flow reserve may be absent⁶⁰. Therefore, these results from normal animals likely cannot be generalized to animals with heart failure.

Effects on the Arterial System

In normal arteries, full support of the normal left ventricle with a continuous-flow LVAD deranged the natural profile of pulsatile arterial hemodynamics. As continuous support increased, arterial diastolic pressure trended upwards, and AoP_{pulse} was reduced by half. In contrast, partial support with a continuous-flow LVAD allowed the aortic valve to open on every beat. A normal profile of cardiac and systemic arterial hemodynamics with a near-normal pulse pressure was preserved.

Potential Mechanism for Unfavorable Histological Changes during Prolonged LVAD Support

Our findings suggest mechanisms by which diminished pulsatility may induce histological changes within the cardiovascular system during prolonged support with a continuous-flow LVAD. When cells in the heart and vasculature are exposed to chronically deranged mechanical forces such as during hypertension, pathological remodeling may occur. For example, chronic hypertension is well documented to induce concentric myocardial hypertrophy in the heart as well as tunica medial thickening and alterations of the collagen/elastin ratio in conduit arteries⁸⁰. During isolated systolic hypertension, pulse pressures are known to exceed 80 to 100 mmHg and affect cardiac and arterial tissues. Even the acute blood pressure elevations that are observed in malignant hypertension may induce acute pathological vascular remodeling that include hyperplastic arteriolosclerosis and fibrinoid necrosis.

During continuous unloading of the cardiovascular system, hemodynamics were similarly deranged but in the opposite direction of hypertension. Full Support reduced the peak left ventricular systolic pressure to a value below the diastolic arterial blood pressure, prevented the aortic valve from opening, and reduced the pulse pressure. As a result, myocardial metabolic demands decreased to values that are not encountered in bovids. Moderate reductions in metabolic demands are likely important, especially if the goal of treatment is myocardial recovery. However, the optimal reduction in cardiac metabolism which allows the heart to rest while maintaining a partial workload has not been established, and excessive myocardial unloading in which the heart performs too little workload may induce myocardial atrophy^{29, 30} and fibrosis^{31, 32}. As a result, it is likely that the lack of adequate myocardial and arterial stretch during full-support in continuous-flow LVAD patients may induce architectural changes in cardiovascular tissues that have long-term consequences, especially if the goal is myocardial recovery. By maintaining pulsatility and a reduced cardiac workload, partial support may attenuate these effects.

In summary, partial support with a continuous-flow LVAD is feasible and may preserve a more normal cardiac and systemic arterial hemodynamic profile. Effects of partial support during heart failure are unlikely to be the same as in normal animals, and the clinical utility of partial support remains to be determined.

CHAPTER V

Aim #2: DEVELOPMENT OF A BOVINE MODEL OF CHRONIC, ISCHEMIC HEART FAILURE

I. Introduction

The development of a stable and reproducible model of chronic heart failure with a low mortality rate is critical for successful translational research on the clinical treatment of heart failure. A large-animal model which closely mimics the human cardiovascular condition (bovine or ovine)⁸¹ is necessary for the preclinical testing of implantable devices as well as to examine (patho)physiological processes associated with mechanical circulatory support. In terms of anatomical size of the thorax and physiological function of the cardiovascular system, normal calves have been used for decades as the industry standard to test safety, performance, and reliability of LVAD systems—phylogenetically lower species are too small to accommodate implantable cardiac devices. Yet large-animal models of *chronic* cardiac pathology have only been used to a limited extent to investigate mechanical circulatory support⁸²⁻⁸⁴ and adjunct therapies⁸⁵ and have not been

used to determine efficacy or to compare the physiological benefits of different strategies of mechanical circulatory support. As a result, mechanisms of reverse myocardial remodeling, myocardial recovery, and pathophysiological responses to mechanical circulatory support such as ischemic and hemorrhagic stroke⁸⁶, acquired von Willebrand disease^{87, 88}, diastolic hypertension^{57, 58}, and gastrointestinal arteriovenous malformations and bleeding⁸⁹ are largely uncharacterized.

Rapid-pacing, volume overload, pressure overload, coronary ligation, microembolization, and cardiotoxic drugs have been used to induce chronic heart failure in multiple species⁹⁰. Additionally, spontaneous genetic cardiomyopathy exists in Holstein-Friesian cattle^{91, 92}. Yet, models of chronic, experimental bovine heart failure^{83, 93} are infrequent in the literature.

Ischemic cardiomyopathy is the most prevalent etiology of heart failure in human patients and currently affects more than 3 million people in the United States alone⁹⁴. As such, large-animal models of chronic, ischemic heart failure are necessary to translate basic discoveries into clinical utility. Coronary microembolization models of ischemic heart failure have been developed and validated in sheep⁹⁵⁻⁹⁹ and in dogs¹⁰⁰. In these studies, 50 to 120 μm spheres injected into the coronary arteries lodged within pre-capillary arterioles, produced myocardial ischemia distal to the lodged spheres, and induced a severe, global left ventricular dysfunction. Over the course of days to weeks, animals developed a stable and reproducible state of left ventricular failure.

Similarly, a bovine model of ischemic heart failure is urgently needed for preclinical studies of mechanical circulatory support. Notably, there is experimental evidence for the feasibility of such an approach. Injection of large quantities of 6 to 14 μm plastic microspheres into the left main coronary arteries in calves resulted in elevated $\text{LVP}_{\text{end diastolic}}$, reduced SV, CO, mean ejection rate, and left ventricular ejection time, and produced widespread microinfarcts through the left ventricle⁹³. Similarly, daily injection of 37 to 70 μm polystyrene microspheres into the left coronary artery of 5 calves resulted in diminished cardiac function, marked reductions in CO, and significant increases in LAP⁸³.

To study acute and chronic effects of LVADs on clinical, functional, histological, and molecular markers of myocardial remodeling during heart failure and mechanical circulatory support, Aim #2 was developed to characterize a bovine model of chronic, ischemic left ventricular failure. Clinically relevant heart failure was defined by a reduction of ejection fraction by greater than 40%, changes in ventricular geometry, hemodynamic imbalance, end-organ hypoperfusion, and histopathological myocardial changes. Results will assist in guiding future investigation into effects of acute and chronic mechanical circulatory support in a large-animal model of chronic cardiac pathology.

The utility of this model extends beyond this project and our laboratory. Our results contribute a novel and clinically relevant, large-animal heart failure model for other investigators, government agencies, and industry.

II. Materials and Methods

Animals

Male Jersey, K-bar, and mixed-breed calves (n=15, 96±8 kg) were used. All animals received humane care and were handled in accordance with National Institutes of Health and University of Louisville animal care committee guidelines. Experimental procedures followed the University of Louisville Institutional Animal Care and Usage Committee approved protocol #09080.

Anesthesia

Twenty-four hours prior to coronary microembolization, oral Atenolol (50 mg) was administered to decrease post-microembolization mortality as previously reported⁹⁵. Animals were pre-anesthetized with Atropine (30 mg) and prepared for strict aseptic surgery. Prophylactic intravenous Cefazolin (15 to 20 mg/kg) was administered. In the catheterization laboratory, general anesthesia was administered with Isoflurane (3-5%) and room air. Tidal volume and respiratory rate were adjusted to maintain arterial oxygen saturation above 90%. An intravenous catheter was placed in the marginal ear vein for administration of normal saline (5 to 10 ml/kg/hour) to maintain arterial blood pressure. An intraarterial catheter was placed in the marginal ear artery to monitor arterial blood pressure. Surface electrocardiographic leads were sutured to the skin in a lead II configuration. Via modified Seldinger technique, a 7-French introducer sheath was introduced into the left common carotid artery. The animal was anticoagulated with a single bolus of intravenous Heparin (100 units/kg).

Cardiac Catheterization and Baseline Hemodynamics

Under fluoroscopic guidance, a 6-French dual-tip pressure-volume conductance catheter (Millar Instruments, Houston, TX) was advanced from the carotid artery into the left ventricle for simultaneous measurement of left ventricular and aortic blood pressures. A 30 second continuous data set was recorded at normal baseline. Approximately 45 minutes after coronary microembolization, this procedure was repeated and a post-embolization heart failure baseline data set was recorded.

Cardiac Catheterization and Baseline End-Organ Blood Flow Measurement

Under fluoroscopic guidance, a 6-French pigtail catheter was advanced from the carotid artery into the left ventricle for injection of 15 μ m fluorescent-labeled microspheres. A single color of fluorescent-labeled microspheres (5.25 million microspheres) was injected into the left ventricular chamber. Simultaneously, a reference blood sample was withdrawn from the carotid sheath at a rate of 15 ml/min for 100 seconds. The microsphere technique enables the precise measurement of regional blood flow in vascular beds of interest as previously described in Chapter IV, Section II.

Coronary Catheterization and Microembolization

Intravenous Lidocaine (100 mg) and Amiodarone (150 mg) were administered to prevent arrhythmia. Under fluoroscopic guidance, a 6-French diagnostic catheter (AL-2) was advanced from the carotid artery to selectively engage the left main coronary artery. Correct catheter placement was confirmed by coronary angiography with radio-opaque

contrast dye (Iothalamate Meglumine, Conray 43, Mallinckrodt, St. Louis MO) as needed.

To induce global left ventricular ischemia and chronic, ischemic heart failure, 90 μm polystyrene microspheres suspended in contrast dye were injected into the left main coronary artery. Real-time observation of electrocardiographic ST-segment changes were observed and confirmed myocardial ischemia, which accompanied hemodynamic impairment (**Figure 4**).

Figure 4: During a typical microembolization session, 90 μm microspheres injected into the left main coronary artery produced electrocardiographic changes characteristic of myocardial ischemia. After 45 minutes, ST-segment elevation and hyperacute, biphasic T-waves indicated severe ischemic myocardial injury. These changes were accompanied by a 25 mmHg decrease in mean arterial blood pressure that persisted at 60 days and indicated chronically impaired myocardial functional capacity. ECG, electrocardiogram

Over the course of 30 to 45 minutes, multiple small boluses of microspheres were injected until severe and sustained electrocardiographic changes were accompanied by impaired hemodynamics. To maintain adequate end-organ blood flow, animals were supported with continuous infusion of intravenous Phenylephrine, Dobutamine, and normal saline to effect. Animals typically exhibited a 10 to 15 mmHg rise in $LVP_{\text{end diastolic}}$. As such, prior to awakening an intravenous bolus of Lasix (80 mg) was administered to promote diuresis, mitigate pulmonary effects of elevated left ventricular filling pressures, and promote recovery and survival.

At the end of the procedure, catheters and sheaths were removed, and percutaneous access sites were closed and compressed for 30 minutes until hemostasis was achieved. Animals were recovered.

Clinical Management

Heart rate, respiratory rate, blood pressure, and ECG were monitored for 48 to 72 hours post-embolization. A constant rate infusion of intravenous Amiodarone (0.5-1.5 mg/min) was maintained for 12 to 48 hours to prevent tachyarrhythmias. A constant rate infusion of intravenous Nitroglycerine (800 µg/hour) was administered for 24 to 48 hours post-embolization. Oral Atenolol (100 mg) was continued twice daily for three days. Intravenous Cefazolin (15 to 20 mg/kg) was administered for 48 to 72 hours post embolization. Arterial blood gases and electrolytes were monitored every 2 to 4 hours for abnormalities. Most animals received intravenous Lasix (0.5 to 2 mg/kg) for respiratory comfort every 4 to 6 hours based on the animals respiratory pattern and effort.

Surface ECG leads were removed when the constant rate infusions were discontinued. In cases of severe heart failure, oral Lasix (0.5 to 4.0 mg/kg) was continued twice daily to reduce pulmonary edema and respiratory distress. Blood draws were used to monitor electrolyte and hydration status.

Echocardiography

Transthoracic echocardiograms were measured at baseline, weekly for 60 days during the progression of heart failure, and while anesthetized during an acute terminal study with the chest open. A Phillips iE-33 machine with S8-3 ultrasound probe was used to obtain 2-dimensional echocardiographic recordings. Parasternal views were used to obtain images. Left ventricular ejection fraction, and end-systolic and end-diastolic volumes were determined from the apical four-chamber view with a modified Simpson technique (summation of discs). Target left ventricular dysfunction was defined as a reduction in left ventricular ejection fraction greater than 40%.

Terminal Study

Sixty days after microembolization, a terminal study was performed to measure the hemodynamic severity of heart failure and to harvest tissues for histology and molecular analyses. Anesthesia was induced and maintained as described in Chapter IV, Section II. The animal was placed on the operating table in right lateral recumbency. Fluid-filled arterial and venous catheters were placed in the left carotid artery and jugular vein for blood sampling. A left thoracotomy was performed. Ribs #4 and #5 were resected. The pericardium was opened. An open-chest echocardiogram was performed.

A single-tip, high-fidelity micromanometer catheter (Millar Instruments, Houston, TX) was placed in the aorta and a dual pressure-volume conductance catheter (Millar Instruments, Houston, TX) was advanced from the left atrium across the mitral valve into the left ventricle for simultaneous measurement of aortic, left atrial, and left ventricular blood pressures. A transit-time ultrasonic flow probe (Transonics, Ithaca, NY) was placed around the pulmonary artery to measure cardiac output. A silicone catheter (7-French, Access Technologies, Skokie, IL) was implanted in the left atrial appendage chamber for injection of 15 μm fluorescent-labeled polystyrene microspheres. The depth and angle of catheter entry parallel to the surface of the left atrial appendage ensured that the catheter did not interfere with mitral valve function.

Experimental Design

In each animal, blood pressure and flow waveforms were recorded for 30 seconds to determine the hemodynamic severity of heart failure. A single color of fluorescent-labeled microspheres (5.25 million microspheres) was injected into the left atrial catheter. Simultaneously, a reference blood sample was withdrawn from the arterial line at a rate of 15 ml/min for 100 seconds. The microsphere technique enabled the precise measurement of regional blood flow in vascular beds of interest as described in Chapter IV, Section II.

Quantification of Microspheres and End-Organ Blood Flow

At the completion of the study, while under anesthesia, euthanasia was performed with a fatal intravenous bolus injection of Beuthanasia-D Special (1 ml/5 kg). The heart was removed and photographed. The ventricles were sectioned into a multi-level map (**Figure 7**). One to two gram tissue sections from the left ventricular free wall, right ventricular free wall, interventricular septum, left ventricular epicardium, mid-myocardium, and endocardium, kidney, liver, spleen, small bowel, lung, skin, skeletal muscle, brainstem, cerebellum, and frontal lobe were collected. Tissue samples and reference blood samples were sent to IMT/Stason Laboratories (Irvine, CA) for automated digestion and counting of fluorescent microspheres using flow cytometry and calculation of tissue specific blood flows in ml/min/g of tissue. Regional myocardial blood-flow maps were constructed on a piece-by-piece basis.

Histology

Triphenyltetrazolium chloride (TTC) staining was performed on gross cross-sections of hearts to approximate the amount of infarcted myocardium. Microscopic myocardial histology was performed on left ventricular and right ventricular samples obtained from heart failure animals (n=9) and compared to myocardium from normal control animals (n=8). Paraffin-embedded tissues were sectioned at 4 μ m, de-paraffinized, rehydrated, and stained. Regional myocardial fibrosis was quantified with Masson's trichrome staining as the ratio of area occupied by collagen stain to the area of myocardium sampled¹⁰¹. Regional myocyte size was determined with FITC-conjugated wheat germ agglutinin and DAPI (Molecular Probes, Invitrogen, Carlsbad, CA) nuclear co-staining to

delineate the cell membrane in 100 cross-sectional cells with centrally located nuclei¹⁰². Regional total cardiac apoptosis was determined with the DeadEnd Fluorometric TUNEL System (Promega, Madison, WI), which catalytically incorporates fluorescein-12-dUTP at DNA strand breaks in cells actively undergoing programmed cell death¹⁰². Nuclei were counterstained with DAPI. Myocyte-specific apoptosis was determined with a combination of Texas Red-conjugated wheat germ agglutinin, TUNEL, and DAPI staining. Images were viewed with epifluorescence microscopy (Nikon TE2000) and analyzed with Metamorph Imaging Software. For each histological stain, a minimum of three fields were analyzed from each myocardial region.

Data Reduction

All transducers were pre- and post-calibrated against known physical standards to ensure measurement accuracy. Calibration curves for the volume conductance catheter were constructed using static and dynamic tests pre- and post-experiment. Hemodynamic data were collected at 400 Hz, signal conditioned, and A/D converted for digital analysis using our GLP compliant data acquisition system⁷⁵.

To determine the hemodynamic severity of heart failure, pressure and flow waveforms were used to derive HR, $LVP_{\text{end diastolic}}$, $LVP_{\text{peak systolic}}$, $\pm dp/dt$, AoP_{mean} , AoP_{systolic} , $AoP_{\text{diastolic}}$, AoP_{pulse} , $HR \times LVP_{\text{peak systolic}}$, PAF as an index of CO, and CO/animal weight. Hemodynamic indices were calculated on a beat-to-beat basis for each 30 second data set with the Hemodynamic Evaluation and Assessment Research Tool (HEART) program⁷⁷ developed in Matlab (Version 6.5, MathWorks, Natick, MA). All analyzed beats in each

data set (approximately 30 to 50 beats/30 second data set) were averaged to obtain a single representative mean value for each calculated variable.

Statistics

GraphPad, version 4.00 (Prism, La Jolla, CA) was used to perform statistical analyses and plot data. One-way repeated measures ANOVA with Tukey post-test was performed to compare weekly echocardiographic measurements. Unpaired student t-tests were performed to compare acute hemodynamics, chronic hemodynamics, end-organ blood flow, and histological findings between heart failure animals and normal animals (n=9)¹⁵. Paired student t-tests were used to compare histological findings between the left and right ventricles within normal control and heart failure animals. All analyses were two-tailed, and a p-value<0.05 (95% confidence) was considered statistically significant. All data are presented as mean±standard error.

III. Results

Survival, Clinical Findings, and Data Collected

Fifteen animals underwent selective left main coronary artery microembolization.

Hemodynamics in 12 animals were studied at baseline prior to coronary microembolization and 45 minutes after microembolization. Nine animals survived the initial embolization period. These animals developed tachycardia, arrhythmias, murmurs, tachypnea, chronic coughing, and dyspnea on exertion. Serial echocardiography was performed weekly until termination. Of the nine animals, eight survived 60 days at which time an elective, terminal study was performed to measure the hemodynamic severity of heart failure, measure end-organ blood flow, and collect tissues.

Echocardiography

Target severity of chronic heart failure (approximately 40 % reduction in ejection fraction) was achieved in eight animals. **Figure 5** demonstrates M-Mode images at baseline (**A**) and 60 days after left main coronary microembolization (**B**) in the same animal. Increased heart rate, hypokinetic left ventricular wall motion, and marked dilatation of the left ventricular chamber are noted.

Left ventricular ejection fraction decreased in all animals (**Figure 5 C**) from a baseline conscious value of $81 \pm 5\%$ to a final conscious value of $43 \pm 13\%$ ($p < 0.01$ vs. baseline) and final anesthetized value of $29 \pm 10\%$ ($p < 0.05$ vs. final conscious). Left ventricular end-diastolic and end-systolic volumes increased in all animals (**Figure 5 D**) from

baseline values of 115 ± 45 ml and 22 ± 10 ml to final values of 173 ± 28 ml ($p < 0.05$ vs. baseline) and 101 ± 53 ml ($p < 0.01$ vs. baseline), respectively.

Figure 5 A, B: M-mode images at baseline and 60 days after coronary microembolization in the same animal. Left ventricular dilatation and systolic dysfunction are present. **C:** Coronary microembolization reduced the left ventricular ejection fraction by approximately 50%. **D:** End diastolic and end-systolic left ventricular dilatation was present.

Acute and Chronic Hemodynamics

Table 5 demonstrates hemodynamic variables measured at baseline and 45 minutes after left main coronary microembolization. Hallmark hemodynamic changes associated with systolic dysfunction were noted despite aggressive pharmacological support of hemodynamics. A significant increase in $LVP_{\text{end diastolic}}$ ($p < 0.0001$) was accompanied by a significant decrease in $-dP/dt$ ($p = 0.04$) and trends toward decreased $LVP_{\text{peak systolic}}$ ($p = 0.07$), AoP_{mean} ($p = 0.14$), AoP_{systolic} ($p = 0.09$), and $AoP_{\text{diastolic}}$ ($p = 0.20$).

Table 6 demonstrates hemodynamic variables measured at 60 days after the induction of heart failure. When compared to normal calves of smaller size (n=9, mean: 76.1 kg)¹⁵, calves that underwent coronary microembolization exhibited hemodynamic changes associated with left ventricular dysfunction. A trend toward a compensatory tachycardia accompanied significantly decreased LVP_{peak systolic} (p=0.008), -dP/dt (p<0.001), AoP_{mean} (p<0.002), AoP_{systolic} (p<0.002), AoP_{diastolic} (p=0.004), and CO/weight (p<0.001). A change in LVP_{end diastolic} did not occur and may have been due to ongoing diuresis used to treat respiratory distress associated with pulmonary edema.

	Baseline (n=12)	Post-Embolization (45 min) (n=12)	p-value
HR (bpm)	61±4	66±3	0.37
LVP _{end diastolic} (mmHg)	15±1	23±2	<0.0001
LVP _{peak systolic} (mmHg)	106±8	95±3	0.07
+dP/dt (mmHg/s)	1,116±230	930±70	0.35
-dP/dt (mmHg/s)	-1,890±243	-1,295±66	<0.05
AoP _{mean} (mmHg)	90±9	80±4	0.14
AoP _{systolic} (mmHg)	106±8	94±4	0.09
AoP _{diastolic} (mmHg)	76±9	66±5	0.20
AoP _{pulse} (mmHg)	31±2	28±2	0.93
HR x LVP _{peak systolic} (bpm x mmHg)	6,567±824	6,306±448	0.74

Table 5: Hemodynamics 45 minutes post-embolization. HR, heart rate; LVP, left ventricular pressure; AoP, aortic pressure

	Baseline (n=9)	Heart Failure (60 Days) (n=8)	p-value
HR (bpm)	78±4	88±7	0.24
LVP _{end diastolic} (mmHg)	16±2	17±2	0.68
LVP _{peak systolic} (mmHg)	103±3	83±6	<0.01
+dP/dt (mmHg/s)	1,252±144	1,193±195	0.80
-dP/dt (mmHg/s)	-2,527±306	-984±95	<0.001
AoP _{mean} (mmHg)	90±4	62±7	<0.01
AoP _{systolic} (mmHg)	105±3	78±7	<0.01
AoP _{diastolic} (mmHg)	73±4	51±6	<0.01
AoP _{pulse} (mmHg)	32±2	28±3	0.22
HR x LVP _{peak systolic} (bpm x mmHg)	8,014±540	7,174±586	0.29
CO (L/min)	8.1±0.4	7.4±1.0	0.47
Weight (kg)	78±4	130±14	<0.01
CO/Weight (ml/min/kg)	105±5	61±9	<0.001

Table 6: Hemodynamics 60 days post-embolization. HR, heart rate; LVP, left ventricular pressure; AoP, aortic pressure; CO, cardiac output

End-Organ Blood Flow

Figure 6 demonstrates end-organ blood flow in normal and chronic heart failure animals.

Reduced blood flow was noted in nine of ten vascular beds studied. Blood flow reductions were quantitatively large in the kidneys, lung, brainstem, and frontal lobe, but were not statistically different in any organ.

Figure 6: Sixty days after coronary microembolization, end-organ blood flow decreased systemically. However, blood flow reductions were not statistically significant in any organ and may have indicated that adequate compensation enabled animals to maintain end-organ perfusion in the lower range of normal.

Regional Myocardial Blood Flow

Figure 7 demonstrates gross heart sections and a multi-level, regional myocardial blood flow map in one animal 60 days after microembolization (**A-C**). This animal received the majority of the dose of microspheres in the left circumflex coronary artery. As such, a large superior and posterolateral myocardial infarction was noted in the gross heart specimen. Small infarcts are noted dispersed throughout the left ventricular free wall and interventricular septum.

The myocardial flow maps and regions of reduced myocardial blood flow corresponded well with the area of gross infarction. Regional right ventricular and anterior interventricular blood flow was largely unaffected.

Global myocardial hypoperfusion was noted (**Figure 7 D, E**). Left ventricular free wall blood flow decreased significantly from control values in normal animals of 0.79 ± 0.09 ml/min/g to 0.34 ± 0.05 ml/min/g ($p < 0.01$) in heart failure animals. Right ventricular free wall blood flow tended to decrease from control values in normal animals of 0.69 ± 0.08 ml/min/g to 0.50 ± 0.10 ml/min/g ($p = 0.17$) in heart failure animals. Similar reductions were noted in the epicardium ($p = 0.10$), mid-myocardium ($p < 0.01$), and endocardium ($p < 0.01$).

Figure 7 A-C: TTC-stained gross heart sections and a multi-level, regional myocardial blood flow map in one animal demonstrated a large superior and posterolateral myocardial infarction. The dose of microspheres was injected selectively into the left circumflex coronary artery. **D, E:** Global myocardial hypoperfusion was noted in heart failure animals versus normal control animals.

Histology

Figure 8 demonstrates the gross and microscopic appearances and percentage of fibrosis in the left and right ventricles in control and microembolized hearts stained with TTC and Masson's Trichrome, respectively. The heart of a control animal appeared uniform in color (**Top Left Panel**). The left ventricular chamber was small.

One week after left main coronary microembolization, the left ventricle exhibited mild dilatation and large and diffuse islands of unconsolidated coagulative necrosis (**Middle Left Panel**). The right ventricle appeared normal.

Eight weeks after coronary microembolization, the left ventricle exhibited marked dilatation with free-wall thinning and consolidation of infarcted myocardium (**Bottom Left Panel**). Although the right ventricular wall appeared grossly normal, marked right ventricular dilatation was noted.

Microembolized left ventricular myocardium contained more fibrosis than normal, control left ventricle ($39 \pm 10\%$ vs. $4 \pm 2\%$, $p < 0.0001$). Right ventricular myocardium in microembolized animals also contained more fibrosis than normal, control right ventricle ($11 \pm 5\%$ vs. $6 \pm 2\%$, $p < 0.05$). In microembolized hearts, left ventricular myocardium contained greater fibrosis than right ventricular myocardium ($p < 0.001$).

Figure 8, Left: Microembolized myocardium exhibited significant coagulative necrosis at 1 week, and consolidated infarction after 8 weeks. **A:** Right ventricular myocardium from a microembolized heart exhibited minimal fibrosis. **B:** Left ventricular myocardium from a microembolized heart exhibited diffuse infarction. **Bar Graph:** Compared to normal control hearts, microembolized hearts contained significantly elevated fibrosis globally. 4X, black bar = 1,000 μm

Figure 9 demonstrates myocyte size in the left and right ventricles in control animals and animals that underwent coronary microembolization. Myocytes in the left ventricle of microembolized hearts were grossly hypertrophic as compared to left ventricular myocytes in normal, control left ventricle (**E vs. C**, $343 \pm 99 \mu\text{m}^2$ vs. $146 \pm 70 \mu\text{m}^2$, $p < 0.01$) and right ventricular myocytes in the same animal (**E vs. D**, $343 \pm 99 \mu\text{m}^2$ vs. $205 \pm 104 \mu\text{m}^2$, $p < 0.05$). Myocytes in the right ventricle of microembolized hearts were also hypertrophic as compared to normal, control right ventricle (**D vs. B**, $205 \pm 104 \mu\text{m}^2$ vs. $91 \pm 28 \mu\text{m}^2$, $p < 0.01$).

Figure 9 A: Significant myocyte hypertrophy was observed in the left and right ventricles of microembolized hearts as compared to normal control animals. Within microembolized hearts, left ventricular myocytes were significantly larger than right ventricular myocytes. **B, C:** Right and left ventricle from normal control animals. **D, E:** Right and left ventricle 60 days after coronary microembolization. 20X, white bar = 200 μm

Figure 10 demonstrates the incidence of total cardiac cell apoptosis in the left and right ventricles in control and microembolized hearts. The total apoptosis rate in the left and right ventricles of microembolized hearts was markedly increased as compared to normal, control left and right ventricle (left ventricle: $0.63 \pm 0.41\%$ vs. $0.12 \pm 0.10\%$, $p < 0.01$; right ventricle: $0.45 \pm 0.34\%$ vs. $0.14 \pm 0.12\%$, $p < 0.05$). Within microembolized hearts, the apoptosis rate in the left ventricle trended toward an increase as compared to the right ventricle ($p = 0.08$).

Figure 10, A: Total cardiac apoptosis increased significantly in the left and right ventricles of microembolized hearts as compared to normal control hearts. **B, C:** DAPI co-nuclear stain. **D, E:** FITC TUNEL+ stain. **F, G:** DAPI plus FITC overlay. Cyan nuclei indicate TUNEL+ cells. 10X, white bar = 400 μ m

Figure 11 demonstrates the incidence of myocyte-specific apoptosis in the left ventricle of control and microembolized hearts. Myocyte apoptosis was markedly increased in microembolized hearts as compared to normal ($0.63 \pm 0.41\%$ vs. $0.12 \pm 0.10\%$, $p < 0.01$).

Figure 11, A: Myocyte-specific apoptosis increased significantly in microembolized hearts as compared to normal control hearts. **B, C:** Texas Red-conjugated wheat germ agglutinin stain. **D, E:** DAPI co-nuclear stain. **F, G:** FITC TUNEL+ stain. **H, I:** Texas Red plus DAPI plus FITC overlay. Cyan nuclei indicate TUNEL+ cells. **I:** A single myocyte undergoing apoptosis is seen in the inset. In the upper right corner, a small nest of TUNEL+ cells are not myocytes and may be inflammatory cells in the extracellular matrix that are participating in ischemic, inflammatory-mediated remodeling. 20X, white bar = 200 μ m

IV. Conclusions

We have developed a novel bovine model of chronic, ischemic heart failure.

Specifically, injection of 90 μm microspheres into the left main coronary artery produced: 1) acute electrocardiographic changes consistent with severe ischemic myocardial injury, 2) a clinical profile consistent with left ventricular failure, 3) acute and chronic hemodynamic changes associated with systolic dysfunction, 4) a sustained and stable 50% reduction in left ventricular ejection fraction, 5) left ventricular dilatation, 6) reduced regional myocardial and systemic end-organ blood flow, 7) 40% fibrosis of the left ventricle, 8) global myocyte hypertrophy, and 9) increased total cardiac and myocyte-specific apoptosis. Our results establish the feasibility and validity of a bovine model of chronic, ischemic heart failure that shares many phenotypic similarities with human heart failure.

In summary, the calf is the industry standard for preclinical testing of cardiac assist devices. As such, this clinically relevant model is an appropriate platform to test acute and chronic (patho)physiological responses to LVADs and other translational therapies. This model may prove useful to develop support strategies to specifically promote reverse myocardial remodeling and myocardial recovery during prolonged mechanical circulatory support.

CHAPTER VI

Aim #3: PARTIAL vs. FULL SUPPORT OF THE FAILING LEFT VENTRICLE WITH A CONTINUOUS-FLOW LVAD

I. Introduction

If combined, the benefits of a full-support device implanted with a minimally invasive surgical approach may expand the target patient population for myocardial recovery with an LVAD. Original *in silico* work suggested that partial support of a failing ventricle with a continuous-flow LVAD may increase cardiac output and lower ventricular filling pressures⁶². The greatest hemodynamic benefits were predicted for less-dilated and less-dysfunctional hearts. Computer simulations were validated in an acute bovine model of cardiac dysfunction in which continuous partial support at a rate of 3 L/min decreased left atrial pressure by 6 to 7 mmHg and increased cardiac output by greater than 1 L/min⁶².

As LVADs are miniaturized, limited thoracotomy, subxiphoid access, thoracic keyhole access, placement of surface devices, or percutaneous implantation may permit earlier therapy in less sick patients. Minimally invasive implantation may increase acceptance

by patients and physicians who are more likely to refer patients for less-invasive surgical therapies⁷¹. As a result, earlier intervention in less-sick patients may increase the public-health impact of mechanical circulatory support. Additionally, these operative approaches often do not require cardiopulmonary bypass¹⁰³. As a result, less postoperative coagulopathy may reduce postoperative bleeding and blood transfusions which play a role in right ventricular dysfunction and infection with LVADs¹⁰⁴. Similarly, early clinical data suggest that partial support does not dramatically increase cardiac output and right ventricular overload and right ventricular failure are unlikely¹⁴.

Recently, a number of partial support LVADs have been developed. For example, the CircuLite Synergy Pocket Micro-Pump (CircuLite Incorporated, Saddle Brook, NJ) is a small continuous-flow LVAD the size of an AA battery (49 mm, outer diameter 14 mm, 25 g)²⁶. The CircuLite LVAD is implanted via a miniature, right-sided thoracotomy without extracorporeal circulation and continuously unloads 2.5 to 3.0 L/min of blood from the left atrium into the subclavian artery.

Less-invasive devices such as the Synergy pump provide important technical benefits. In the event of pump thrombosis, device failure, or pump-pocket infection, the device may be easily and rapidly exchanged through the original infraclavicular incision without entering the chest. An additional and unique advantage is that the implantation procedure involves a right-sided thoracotomy in which it is unlikely that adhesions will form in the anterior mediastinum and complicate median sternotomy if heart transplantation is indicated. A future design that obviates entrance into the thorax will include an inflow

graft placed percutaneously through the subclavian vein and advanced through the interatrial septum into the left atrium. As with the current design, the outflow graft will be sewn to the subclavian artery. With this approach, the need for a thoracotomy will be eliminated, and the infraclavicular incision will be the only incision necessary to implant the entire system¹⁰⁵. This approach will likely further increase acceptance by patients and referring physicians.

Initial results with the CircuLite device are encouraging. In an ongoing multicenter European clinical trial^{14, 26, 105}, ambulatory patients on the transplant list with inotrope-independent NYHA class IIIB or IVA heart failure and preserved end-organ function demonstrated hemodynamic improvements over a 3-month follow-up (ongoing maximum support duration of eight months)^{14, 26, 105}. Partial hemodynamic recovery included significant increases in cardiac index from 2.0 ± 0.4 to 2.8 ± 0.6 L/min/m², an increase in AoP_{mean} from 67 ± 8 to 80 ± 9 mmHg, and a reduction in pulmonary capillary wedge pressure from 30 ± 5 to 18 ± 5 mmHg²⁶. N-terminal fragment pro-brain natriuretic peptide was significantly reduced from $6,452 \pm 5,470$ to $3,209 \pm 2,379$ pg/ml¹⁰⁵ and suggested a reduction in myocyte stress. As predicted, right heart failure was not a clinical challenge with the CircuLite pump. During support, the acute increase in cardiac output ranged from 1.0 to 1.5 L/min and did not overload the right ventricle¹⁴.

These preliminary results in humans suggest that partial support is not only safe but may also be an effective strategy to support less dysfunctional hearts. These findings encourage basic scientific investigation to further validate and improve this approach.

For example, in the current European trial, implants of the CircuLite pump were performed primarily to demonstrate long-term safety and efficacy as a bridge-to-transplant therapy. As such, this device has not yet been implanted with the specific goal of myocardial recovery.

Accordingly, experimental studies will be necessary to define the clinical utility of partial circulatory support for the treatment of early stages of heart failure. A major undertaking will be to evaluate whether a protocol that includes partial unloading may promote myocardial recovery and in which patient population(s). As a first step, it will be important to understand how the left ventricle should be unloaded in preparation for prolonged partial support. The major goal of Aim #3 was to examine acute responses to partial vs. full support of the failing left ventricle with a continuous-flow LVAD. The effect of continuous unloading on the myocardial supply/demand relationship was studied in animals with chronic, ischemic heart failure. Standard indices of cardiovascular performance and regional myocardial blood flow were measured during each support mode. Implications for reverse myocardial remodeling and myocardial recovery were considered. Results of this study will assist in guiding future investigation into the chronic effects of continuous-flow mechanical circulatory support in large-animal models of chronic cardiac pathology.

II. Materials and Methods

Animals

Male Jersey, K-bar, and mixed-breed calves from Aim #2 that had undergone left main coronary artery embolization and exhibited signs of chronic, ischemic heart failure (n=9, 130±13 kg) were used. All animals received humane care and were handled in accordance with National Institutes of Health and University of Louisville animal care committee guidelines. Experimental procedures followed the University of Louisville Institutional Animal Care and Usage Committee approved protocol #09080.

Anesthesia

Animals were pre-anesthetized with Atropine (30 mg) and prepared for acute, non-sterile surgery. In the operating room, general anesthesia was administered with Isoflurane (3-5%) and room air. The animal was placed on the operating table in the right lateral recumbency. Tidal volume and respiratory rate were adjusted to maintain arterial oxygen saturation above 90%. Fluid-filled arterial and venous catheters were placed in the left carotid artery and jugular vein for blood sampling. A left thoracotomy was performed. Ribs #4 and #5 were resected. The pericardium was opened. The animal was anticoagulated with a single bolus of intravenous Heparin (100 units/kg). For the remainder of the procedure, the ACT was maintained above 250 s with additional boluses of Heparin (1,000 to 2,000 units).

Surgical Instrumentation

A single-tip, high-fidelity micromanometer catheter (Millar Instruments, Houston, TX) was placed in the aorta and a dual pressure-volume conductance catheter (Millar Instruments, Houston, TX) was advanced from the left atrium across the mitral valve into the left ventricle for simultaneous measurement of aortic, left atrial, and left ventricular blood pressures. A transit-time ultrasonic flow probe (Transonics, Ithaca, NY) was placed around the pulmonary artery to measure cardiac output. In six animals, a silicone catheter (7-French, Access Technologies, Skokie, IL) was advanced in the left atrial appendage chamber for administration of 15 μ m fluorescent-labeled polystyrene microspheres. The depth and angle of catheter entry parallel to the surface of the left atrial appendage ensured that the catheter did not interfere with mitral valve function.

LVAD Implantation

A continuous-flow LVAD (HeartWare HVAD n=4, Thoratec HeartMate II n=5) was implanted without cardiopulmonary bypass. The outflow graft was anastomosed to the descending aorta. The left ventricular apex was cored and cannulated. The device and outflow graft were de-aired. A transit-time ultrasonic flow probe (Transonics, Ithaca, NY) was placed around the outflow graft to measure LVAD flow.

Experimental Design

In each animal, blood pressure and flow waveforms were recorded during Heart Failure Baseline (pump off, outflow graft clamped), Low Partial Support (~1.5 L/min support, aortic valve opening every beat), High Partial Support (~3 L/min support, aortic valve

opening every beat), and Full Support (~5 L/min, aortic valve maintained closed, left ventricle maximally unloaded). Heart Failure Baseline and support modes for each device were maintained for 10 minutes each to achieve steady-state conditions prior to collection of 30 second data sets. During each condition, a single color of fluorescent-labeled microspheres (5.25 million microspheres) was injected into the left atrial catheter. Simultaneously, a reference blood sample was withdrawn from the arterial line at a rate of 15 ml/min for 100 seconds. The microsphere technique enabled the precise measurement of regional myocardial blood flow as described in Chapter IV, Section II.

In one animal, ventricular fibrillation occurred prior to the implantation of the LVAD. Open-chest cardiac massage was performed. Multiple shocks were attempted but defibrillation was unsuccessful. However, after implantation and operation of the LVAD at full support for approximately 20 minutes, the animal was successfully defibrillated. The estimated ischemic time was approximately 60 minutes. Prior to data collection, it was noted that substantial ST-segment elevation accompanied a coronary hyperemia. As a result, the regional myocardial blood flow data from this animal was not used.

Quantification of Microspheres and Regional Myocardial Blood Flow

At the completion of the study, while under anesthesia, euthanasia was performed with a fatal intravenous bolus injection of Beuthanasia-D Special (1 ml/5 kg). The heart was removed. One to two gram tissue sections from the left ventricular free wall, right ventricular free wall, interventricular septum, and left ventricular epicardium, mid-myocardium, and endocardium were collected. Myocardial samples and reference blood

samples were sent to IMT/Stason Laboratories (Irvine, CA) for automated digestion, counting of fluorescent microspheres with flow cytometry, and calculation of tissue specific blood flows in ml/min/g of tissue.

Data Reduction

All transducers were pre- and post-calibrated against known physical standards to ensure measurement accuracy. Calibration curves for the volume conductance catheter were constructed using static and dynamic tests pre- and post-experiment. Data were collected at 400 Hz, signal conditioned, and A/D converted for digital analysis using our GLP compliant data acquisition system⁷⁵.

To determine hemodynamic performance during each support mode, pressure and flow waveforms were used to derive LVADF, HR, SV, PAF as an index of CO, LAP, $LVP_{end\ diastolic}$, $LVP_{peak\ systolic}$, $\pm dP/dt$, $HR \times LVP_{peak\ systolic}$, $AoP_{systolic}$, $AoP_{diastolic}$, AoP_{mean} , AoP_{pulse} . Myocardial vascular resistance was calculated as $AoP_{mean}/region-specific$ myocardial blood flow. Hemodynamic variables were calculated on a beat-to-beat basis for each 30 second data set with the Hemodynamic Evaluation and Assessment Research Tool (HEART) program⁷⁷ developed in Matlab (Version 6.5, MathWorks, Natick, MA). All analyzed beats in each data set (approximately 30 to 50 beats/30 second data set) were averaged to obtain a single representative mean value for each calculated variable.

Statistics

GraphPad, version 4.00 (Prism, La Jolla, CA) was used to perform statistical analyses and plot data. One-way repeated measures ANOVA with Tukey post-test was performed for each hemodynamic index, region of myocardial blood flow, and myocardial blood flow normalized to $HR \times LVP_{\text{peak systolic}}$ to compare Heart Failure Baseline, Low Partial Support, High Partial Support, and Full Support modes within each animal. An unpaired student t-test was performed to compare the myocardial blood supply/demand ratio between normal and heart failure animals at baseline and during each level of support. All analyses were two-tailed, and a p-value<0.05 (95% confidence) was considered statistically significant. All data are presented as mean±standard error.

III. Results

Table 7 demonstrates that in animals with chronic heart failure, as the level of support with a continuous-flow LVAD increased, the cardiac and systemic arterial hemodynamic profile progressively changed. An increase in continuous support increased CO in a dose-dependent manner. The observed increases were small and were not quantitatively different between support modes. HR did not change during LVAD support.

Left Ventricular Hemodynamics

As in normal animals, left ventricular pressures demonstrated the most robust changes. The progressive increase in continuous support significantly decreased LAP ($p=0.02$), $LVP_{\text{end diastolic}}$ ($p<0.01$), and $LVP_{\text{peak systolic}}$ ($p<0.01$) with a dose-dependent response. Reductions were greatest and indicated maximum unloading during Full Support. Smaller reductions were observed during partial-support modes. Full Support significantly reduced $+dP/dt$ ($p<0.001$) and $-dP/dt$ ($p=0.03$). However, partial-support modes did not affect $\pm dP/dt$.

As seen in normal animals, during Full Support the variation between end-systolic and end-diastolic pressures decreased to non-physiologically low values and resulted in chronic closure of the aortic valve. In this situation, the average $LVP_{\text{peak systolic}}$ (57 ± 9 mmHg) did not exceed the $AoP_{\text{diastolic}}$ (67 ± 6 mmHg). As a result, the aortic valve remained closed, and $HR \times LVP_{\text{peak systolic}}$ decreased significantly ($p<0.001$) to values that are not seen in normal animals. This finding indicated that unloading the heart with a

continuous-flow LVAD dramatically reduced myocardial metabolic demands and with a response dependent on the degree of support.

Arterial Hemodynamics

Systemic arterial pressures exhibited more robust changes than were observed in normal animals. As continuous support increased, $AoP_{systolic}$ did not change. However, $AoP_{diastolic}$ increased significantly by 16 mmHg ($p<0.001$) and AoP_{mean} trended toward an increase of 8 mmHg ($p=0.11$). AoP_{pulse} did not change during Low Partial Support. However, during High Partial Support, AoP_{pulse} decreased significantly from 27 ± 3 to 15 ± 2 mmHg ($p<0.0001$), which was comparable to the reduction observed in normal animals during Full Support. Importantly, during Full Support, the arterial pulse pressure was significantly reduced from 27 ± 3 to 5 ± 1 mmHg ($p<0.0001$). In contrast, during Low Partial Support, AoP_{pulse} did not change from Heart Failure Baseline.

Myocardial Blood Supply/Demand Relationship

In animals with chronic heart failure, continuous support of the left ventricle did not affect right ventricular myocardial vascular resistance or blood flow. However, in the left ventricle and interventricular septum, increasing levels of continuous support significantly reduced the cardiac workload, and increased the myocardial vascular resistance (**Table 8**) with a dose-dependent response. However, the increase in vascular resistance was not as pronounced as in normal animals, and regional left ventricular

n=8	LVADF (L/min)	HR (bpm)	LV SV (ml)	PAF (L/min)	LAP (mmHg)	LVP _{end diastolic} (mmHg)	LVP _{peak systolic} (mmHg)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)	HR x LVP _{peak systolic} (bpm x mmHg)	AoP _{mean} (mmHg)	AoP _{systolic} (mmHg)	AoP _{diastolic} (mmHg)	AoP _{pulse} (mmHg)
ANOVA p-value	<0.0001	0.94	<0.0001	0.19	0.02	<0.01	<0.001	<0.001	0.03	<0.001	0.13	0.37	<0.001	p<0.0001
Heart Failure Baseline	0.0±0.0	86±8	89±12	7.3±0.9	16±2	19±3	84±6	1,092±166	-1,054±160	7,013±466	62±7	78±7	51±6	27±3
Low Partial Support	2.3±0.3*	86±7	66±11*	7.7±1.1	13±2	16±2	84±4	1,106±216	-1,050±96	7,068±500	67±5	80±5	57±5	22±2
High Partial Support	3.7±0.4*†	85±9	48±9*	7.6±1.1	13±3	15±2	83±6	1,007±185	-973±140	6,902±629	71±7	79±8	64±6*	15±2*†
Full Support	6.2±1.1*†‡	85±7	20±5*†‡	7.9±1.3	10±2*	12±2*	57±9*†‡	606±68*†‡	-635±137*†	4,558±428*†‡	70±6	73±7	67±6*†	5±1*†‡

Table 7: Hemodynamics during partial vs. full support of the failing left ventricle with a continuous flow LVAD. LVADF, left ventricular assist device flow; HR, heart rate; LV SV, left ventricular stroke volume; PAF, pulmonary artery flow; LAP, left atrial pressure; LVP, left ventricular pressure; AoP, aortic pressure; *p<0.05, support mode vs. Heart Failure Baseline; †p<0.05, support mode vs. Low Partial Support; ‡p<0.05, support mode vs. High Partial Support

blood flow did not change (**Table 9**). Vascular resistance and blood flow in the epicardial region did not change. Likely, the coronaries and large coronary arterioles were less responsive than in normal control animals, in which large increases in epicardial vascular resistance were observed.

n=5	RV	LV	Septum	Epicardium	Mid-Myocardium	Endocardium
	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)	(mmHg/ml/min/g)
ANOVA p-value	p=0.98	p=0.04	p=0.01	p=0.61	p<0.01	p=0.31
Heart Failure Baseline	175±29	164±20	136±22	160±27	140±21	199±52
Low Partial Support	167±25	187±45	141±17	168±29	159±27	197±42
High Partial Support	175±12	206±34	170±17	200±33	172±23	252±63
Full Support	167±13	249±25*	194±24*†	194±24	217±20*†	217±20

Table 8: Regional myocardial vascular resistance during partial vs. full support of the failing left ventricle with a continuous flow LVAD. RV, right ventricle; LV, left ventricle; *p<0.05, Full Support vs. Heart Failure Baseline; †p<0.05, support mode vs. Heart Failure Baseline; ‡p<0.05, support mode vs. Low Partial Support

n=5	RV	LV	Septum	Epicardium	Mid-Myocardium	Endocardium
	(ml/min/g)	(ml/min/g)	(ml/min/g)	(ml/min/g)	(ml/min/g)	(ml/min/g)
ANOVA p-value	p=0.81	p=0.85	p=0.13	p=0.83	p=0.18	p=0.48
Heart Failure Baseline	0.40±0.04	0.41±0.04	0.51±0.05	0.41±0.02	0.49±0.05	0.39±0.06
Low Partial Support	0.45±0.06	0.44±0.08	0.52±0.05	0.40±0.06	0.49±0.08	0.40±0.06
High Partial Support	0.43±0.05	0.40±0.08	0.44±0.05	0.39±0.06	0.46±0.08	0.35±0.07
Full Support	0.45±0.03	0.45±0.03	0.40±0.05	0.40±0.05	0.35±0.04	0.32±0.70

Table 9: Regional myocardial blood flow during partial vs. full support of the failing left ventricle with a continuous flow LVAD. RV, right ventricle; LV, left ventricle

To characterize the relationship between myocardial blood supply and demand, regional myocardial blood flow was normalized to $HR \times LVP_{\text{peak systolic}}$ (**Table 10**). During partial support modes, the ratio between left ventricular workload and left ventricular myocardial blood flow did not change. However, during full support the supply/demand ratio improved significantly in the left ventricular free wall (p=0.04) and interventricular septum (p<0.01) to values comparable to control values obtained in normal animals.

Figure 12 summarizes the gross physiological changes observed during continuous unloading of the failing left ventricle.

Comparison to Normal Animals

Without support, the myocardial blood supply/demand relationship in the left ventricle demonstrated lower values in microembolized hearts as compared to normal, control hearts ($p=0.06$). In normal hearts, support with a continuous-flow LVAD did not change this ratio. However, in microembolized hearts, this difference decreased with greater levels of support (Low Partial Support $p=0.12$, High Partial Support $p=0.24$, Full Support $p=0.47$) and indicated normalization of this relationship toward baseline values.

n=5	LV/HRxLVP _{peak systolic} (ml/min/100 g/bpm · mmHg)	Septum/HRxLVP _{peak systolic} (ml/min/100 g/bpm · mmHg)
ANOVA p-value	p=0.04	p<0.01
Heart Failure Baseline	0.0054±0.0007	0.0066±0.0009
Low Partial Support	0.0064±0.0008	0.0077±0.0009
High Partial Support	0.0057±0.0007	0.0066±0.0008
Full Support	0.0079±0.0014*	0.0099±0.0013*‡

Table 10: Regional myocardial blood supply/demand relationship during partial vs. full unloading of the failing left ventricle with a continuous flow LVAD. LV, left ventricle; HR, heart rate; LVP, left ventricular pressure; * $p<0.05$, Full Support vs. Heart Failure Baseline; ‡ $p<0.05$, support mode vs. High Partial Support

	<u>Normal Animals</u>	<u>Heart Failure Animals</u>
Cardiac Workload	↓	↓
Myocardial Vascular Resistance	↑	↑
Myocardial Flow	↓	↔
Flow/Workload	↔	↑

Figure 12: During support of the failing left ventricle with a continuous-flow LVAD, cardiac workload decreased, and myocardial vascular resistance increased with a dose-dependent response. However, myocardial blood flow did not change because diastolic blood pressure was significantly greater during LVAD support and increased the driving force for coronary blood flow. As a result, the ratio between blood supply/demand improved to values observed in normal control animals. These findings suggested that in animals with chronic heart failure, the heart may have been operating at an insufficient ratio of blood supply/demand, and a coronary reserve was not present.

IV. Conclusions

The study of partial vs. full support of the left ventricle with a continuous-flow LVAD was possible in animals with cardiac pathology. Specifically, in animals with chronic, ischemic heart failure, 1) a systemic flow reserve was not present, and any level of support with a continuous-flow LVAD augmented systemic flows, 2) full support with a continuous-flow LVAD deranged pulsatility, whereas partial support preserved a more normal pulsatile profile, 3) full but not partial support of the failing left ventricle with an LVAD normalized the blood supply/demand relationship, 4) divergent results observed in normal animals vs. heart failure animals suggested that mechanical circulatory support studies should be conducted in large-animal models of chronic cardiac pathology. These results will assist to guide future *in vivo* studies to examine myocardial and arterial remodeling during chronic partial vs. full support of the heart with a continuous-flow LVAD.

Systemic Effects

In animals with chronic heart failure, a systemic flow reserve was absent. At any level of support with a continuous-flow LVAD, CO increased and HR did not change. This finding in animals with chronic heart failure was not unexpected and suggested that any level of flow augmentation with a continuous-flow LVAD may improve systemic arterial blood flow. As such, partial-support with a continuous-flow LVAD in patients with chronic heart failure may be a successful therapy for patients with hypoperfused end organs.

Effects on the Heart

In the failing heart, full support with a continuous-flow LVAD deranged the normal profile of pulsatile cardiac hemodynamics. As continuous support increased, the peak left ventricular pressure (57 ± 9 mmHg) dropped below the arterial diastolic pressure (67 ± 6 mmHg). Insufficient preload was available to eject through the aortic valve, which did not open. As a result, the workload of the heart was dramatically reduced to values that are not observed in bovids. As in normal animals, during continuous unloading of the failing left ventricle, cardiac workload decreased, and myocardial vascular resistance increased with a significant dose-dependent response. However, in contrast to normal animals, during full support of animals with chronic heart failure, a significantly increased diastolic blood pressure increased coronary perfusion despite dramatically reduced myocardial metabolic demands. Importantly, the ratio of blood supply/demand improved to values observed in normal control animals. These findings suggested that in animals with chronic heart failure, a coronary reserve was not present and the heart may have been operating at an insufficient ratio of blood supply/demand. The lack of a change in vascular resistance and blood flow in the epicardial region during any level of support further suggested that coronary vasoreactivity was impaired such that coronary vasoconstriction did not occur, thereby ensuring adequate myocardial blood flow.

Partial support provided inadequate reduction of myocardial metabolism and augmentation of diastolic blood pressure to improve the blood supply/demand relationship. As a result, it seems that partial support may be inappropriate as the first step in a multistep protocol to provide prolonged unloading toward myocardial recovery.

Figure 13, Left Myocardial Map: In animals with ischemic heart failure, the left ventricle operated at a reduced myocardial blood supply/demand ratio. **Center Myocardial Maps:** Immediately after the implantation of an LVAD, partial support was ineffective at rebalancing this relationship. **Right Myocardial Map:** However, full support normalized the blood supply/demand relationship throughout the left ventricle and interventricular septum.

Yet, full support of the failing left ventricle with an LVAD did restore a normal myocardial blood supply/demand relationship (**Figure 13**). This novel finding suggests that during the immediate postoperative period after the implantation of an LVAD, the heart should be fully unloaded.

This conclusion should be interpreted with caution. Full support decreased the $HR \times LVP_{\text{peak systolic}}$, an index of myocardial metabolic demand, to values that are not encountered in bovids. In heart failure, moderate reductions in metabolic demands are likely favorable, especially if the goal of treatment is myocardial recovery. However, the optimal reduction in cardiac metabolism which allows the heart to rest while still maintaining a partial workload has not been established, and excessive myocardial

unloading in which the heart performs too little work may induce myocardial atrophy^{29, 30} and fibrosis^{31, 32}.

Nonetheless, full support of the failing left ventricle by a continuous-flow LVAD has resulted in myocardial recovery⁹. Yet, myocardial recovery has most frequently been reported in patients that received pulsatile ventricular assistance^{5, 6}. This critical finding suggests that variations in the ventricular pressure-volume relationship and cardiac workload may be important to reverse myocardial remodeling during heart failure. It is unclear how variations in stroke work affect myocardial remodeling. However, it is known that normal ventricular geometry depends on a consistent physiologic pressure-volume relationship. As we have demonstrated, partial unloading preserved this relationship and permitted ejection through the aortic valve on every beat. These findings suggest a mechanism by which low native-heart volume output and loss of myocardial mechanical stretch may cause the heart to become a stiff and non-functional chamber.

Therefore, it remains to be determined how long temporary full support with a continuous-flow LVAD is necessary before a transition to partial support is safe. Only after interrupting the progressive hemodynamic deterioration of heart failure, normalizing autonomic tone, and restoring normal end-organ perfusion may the heart regain adequate function and be weaned to a partial-support mode. At that time, the reinstatement of a partial cardiac workload may prevent unfavorable remodeling that is characteristic of prolonged complete unloading of the failing left ventricle. Long-term studies in our model of chronic, ischemic heart failure are necessary to determine the length of full

support prior to a transition to partial support that may prevent myocyte atrophy and left ventricular fibrosis.

Effects on the Arterial System and Emerging Novel Pathologies

In the arteries of animals with chronic heart failure, full support of the left ventricle with a continuous-flow LVAD deranged the normal profile of pulsatile arterial hemodynamics. As continuous support increased, arterial diastolic pressure increased significantly, and the AoP_{pulse} collapsed to 5 ± 1 mmHg, a value which is not compatible with life in mammals in nature²⁸. This finding has important implications for prolonged LVAD therapy. Although increased diastolic blood pressure favors an increase in myocardial and end-organ blood flow, chronic non-pulsatile blood flow may alter the structure and physiological reactivity of arteries and arterioles. As the AoP_{pulse} decreases, vascular resistance increases. Indeed, we have reported acute increases in vascular impedance in human patients in response to continuous-flow mechanical circulatory support¹⁰⁶. Acute physiologic studies in canines have demonstrated that systemic vascular resistance is inversely related to AoP_{pulse} and that systemic vascular resistance during non-pulsatile circulation is as much as 134% of that during pulsatile circulation¹⁰⁷. Chronic studies in goats undergoing prolonged, non-pulsatile cardiopulmonary bypass demonstrated atrophic changes in the tunica media of the aorta that included decreased total smooth muscle volume, reduced cell size, changes in the constituent volume ratio of collagen and elastin, and decreased number of myofilaments and chromatin in smooth muscle cells¹⁰⁸. Additional studies in the same model demonstrated decreased arterial vasoconstrictive response to norepinephrine¹⁰⁹ and phenylephrine¹¹⁰ and suggested that

non-pulsatile blood flow affects vascular reactivity. Overall, the net effect of prolonged non-pulsatile blood flow is endothelial remodeling, loss of arterial reactivity, and extracellular matrix remodeling, which alter arterial visco-elastic performance characteristics and produces stiff and unresponsive arteries.

It may be speculated that changes to the endothelium may contribute to novel pathologies observed in patients with prolonged non-pulsatile blood flow. For example, recent reports have documented an increased incidence in refractory diastolic hypertension^{57, 58}, ischemic and hemorrhagic stroke⁸⁶, acquired von Willebrand disease^{87, 88}, and gastrointestinal arteriovenous malformations and bleeding⁸⁹ in patients with continuous-flow LVADs. These cardiovascular pathologies likely share a common etiology related to the absence of pulsatile stretch of arteries and/or the influence of high, pulseless shear stress on the endothelium. Although causality and mechanism remain to be determined, effects of prolonged diastolic hypertension with a narrow pulse pressure on the vasculature (and heart) may be a limiting factor in long-term patient outcomes.

Clinical Implications

These findings have important clinical implications for device development, selection, operation, and weaning. If combined, the benefits of a full-support device implanted with a minimally invasive surgical approach may expand the target patient population for myocardial recovery with an LVAD. As LVADs are miniaturized, limited thoracotomy, subxiphoid access, thoracic keyhole access, placement of surface devices, or percutaneous implantation may permit initial full support which is later reduced to partial

support. Minimally invasive implantation may increase acceptance by patients and physicians who are more likely to refer patients for less-invasive surgical therapies⁷¹. Earlier intervention in less-sick patients may increase the public-health impact of mechanical circulatory support. Additionally, these operative approaches often do not require cardiopulmonary bypass¹⁰³. As a result, less postoperative coagulopathy may reduce postoperative bleeding and blood transfusions which play a role in right ventricular dysfunction and infection with LVADs¹⁰⁴.

In summary, partial support with a continuous-flow LVAD is feasible and may preserve a more normal cardiac and systemic arterial hemodynamic profile. However, partial support may not be appropriate as the initial step in the clinical management of patients with chronic heart failure. Only after a period of full support may the level of support be reduced. Additional studies are needed to determine the duration of full support necessary prior to prolonged partial support.

CHAPTER VII

SUMMARY AND FUTURE RESEARCH DIRECTIONS

I. Summary of Findings

- 1) **Left main coronary artery microembolization induced chronic, ischemic heart failure in calves. A stable and reproducible large-animal model of chronic heart failure is possible with many phenotypic similarities to clinical heart failure.**
- 2) **In normal animals and animals with chronic heart failure, full but not partial support with a continuous-flow LVAD deranged the physiological profile of pulsatile cardiac and arterial hemodynamics.**
- 3) **In normal animals, neither full nor partial support with a continuous-flow LVAD affected the myocardial blood supply/demand relationship. However,**

in animals with chronic heart failure, full but not partial support normalized the myocardial blood supply/demand relationship.

- 4) **Divergent results were observed between normal animals and animals with chronic heart failure. Normal animals do not reproduce the complex pathophysiological presentation of chronic heart failure and are not ideal for validation and proper translation of mechanical circulatory support strategies into clinical practice.**

In order to translate basic scientific findings from the bench into the clinic, an appropriate and clinically relevant large-animal model is necessary. We have developed a bovine model of chronic, ischemic heart failure that reproduces the complex pathophysiological presentation of functional, neurohormonal, and architectural derangements that are typical in chronic heart failure. This model is relevant for preclinical testing of cardiac devices and other translational therapies.

In this model, complete unloading of the failing left ventricle rebalanced the myocardial blood supply/demand relationship. However, full-support with a continuous-flow LVAD dramatically changed cardiac and systemic arterial hemodynamics, which may have long-term consequences such as myocardial and arterial remodeling.

In contrast, partial support was unable to normalize the blood supply/demand relationship. Yet, partial support augmented systemic flows and preserved a normal

profile of pulsatile hemodynamics in which the aortic valve opened on every beat.

Accordingly, why not combine the two modes of support? Our findings suggest that in the immediate postoperative period after the implantation of an LVAD, complete unloading of the failing left ventricle will rebalance the myocardial blood supply/demand relationship. After a period of full support, it may be effective to reduce support and restore native pulsatility.

Chronic studies are necessary to determine whether a transition to partial support may prevent myocardial atrophy and fibrosis that is seen with prolonged full support while still maintaining adequate end-organ blood flow. These studies should not be performed in normal animals. Our bovine model of chronic, ischemic heart failure is appropriate for such a study.

II. *Future Directions*

Recent international experience with continuous-flow devices has progressively improved clinical outcomes and the quality of life of patients that receive mechanical circulatory support. New databanks for implantable cardiac devices such as INTERMACS will increase this trend. As devices are miniaturized for earlier support with less-invasive operative approaches, the incidence and prevalence of long-term mechanical circulatory support is likely to increase globally.

Less-invasive surgical approaches and strategies of long-term partial unloading of the heart must be further investigated and refined. Invasive full-support devices may still be reserved as a final treatment option for patients with life-threatening, end-stage heart failure as a bridge to heart transplantation or as a destination therapy. In contrast, less-invasive partial-support devices may prove successful for the management of less-severe heart failure and relieve heart transplantation waiting lists as a destination therapy or as a bridge to myocardial recovery. Partial-support devices may interrupt the progressive hemodynamic deterioration of heart failure, improve symptoms and quality of life, promote reverse myocardial remodeling, and allow for device explantation in select patients.

With these goals in mind, additional *chronic* studies on effects of full and partial support are needed to investigate mechanisms of pathological remodeling, reverse myocardial remodeling, functional myocardial recovery, and pathophysiological responses to

mechanical circulatory support. The mechanisms of histological changes in the myocardium and conduit arteries are largely unknown. Endothelial cells are affected by altered pulsatility and shear stress, but the role(s) of the endothelium in remodeling processes are unknown. Similarly, angiogenesis is dependent on pulsatility and shear stress and may be affected by systemic flow alterations with a continuous-flow LVAD.

Interactions between the autonomic nervous system and flow alterations with a continuous-flow LVAD have not been defined but likely include altered baroreceptor reflex sensitivity, catecholamine release, and arterial tone. These processes, which are influenced by hemodynamic alterations, may affect end-organ blood flow, functional capacity, and potential for recovery.

Going forward, experiments may be designed in which animals receive prolonged partial or full support (or a combination of the two) with a continuous-flow LVAD. In these studies it will be possible to further understand acute and chronic responses to continuous-flow mechanical circulatory support.

Our model of chronic, ischemic heart failure is an appropriate and clinically relevant heart failure substrate. Chronic studies will assist to further understand basic pulsatile physiology as well as to define the clinical utility of partial support toward myocardial recovery.

Ultimately, proper patient selection will be critical to achieve success with any device. Device therapy should be tailored to patient needs. As such, careful examination of preclinical and clinical experiences with current and future generations of implantable devices will determine the relative utility for different strategies of mechanical circulatory support.

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EDUCATION

UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE

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- 2011 **Doctor of Philosophy, Ph.D.** – Physiology and Biophysics
 Advisor, Sumanth D. Prabhu
 Graduate Dean's Citation
- 2010 **Master of Science, M.S.** – Physiology and Biophysics
 GPA 4.000

HARVARD UNIVERSITY

- 2007 **Master of Liberal Arts, M.L.A.** – Biological Technology
 GPA 3.5000

CORNELL UNIVERSITY

- 2004 **Bachelor of Science, B.S.** – Biology
 Magna Cum Laude
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HONORS & AWARDS

2011	John Richard Binford Memorial Award University of Louisville School of Graduate Studies
2011	Graduate Dean's Citation University of Louisville School of Graduate Studies
2011	Sponsored Research Tuition Award, University of Louisville Department of Physiology and Biophysics
2010	Semifinalist, National TYLENOL Scholarship University of Louisville
2010	1 st Place (Co-recipient), Engineering Collaboration Award, Research! Louisville, University of Louisville
2009	1 st Place, Medical Student Research Award Research! Louisville, University of Louisville
2008	1 st Place, Engineering Collaboration Award, Research! Louisville, University of Louisville
2008	Thomas B. Calhoon Physiology Prize Finalist University of Louisville, School of Medicine
2008	Summer Research Scholar University of Louisville, School of Medicine
2007-2013	MD/PhD Student Fellowship James Graham Brown Cancer Foundation
2007	Summer Research Scholar University of Louisville, School of Medicine
2007	Poster of Honorable Mention Harvard School of Public Health Symposium
2004	<i>Magna Cum Laude</i> Cornell University, Department of Biology
2004	High Honors in Physiology Research Cornell University, Department of Biology
2004	Golden Key Honor Society Cornell University, Class of 2004

2004	Ho-Nun-De-Kah Honor Society Cornell University, Class of 2004
2003-2004	Biology Honors Program Cornell University
2003-2004	AEA National Pre-Medical Honor Society Cornell University
2003	American Heart Association Summer Fellow Cornell University, Department of Biomedical Sciences
2000-2004	Dean's List Cornell University
2000-2001	William Carran Scholar Cornell University
2000	National Cum Laude Society The Williston Northampton School (High School)
1999, 2000	National Advanced Placement Scholar With Distinction
'95, '98, '99	<i>Maxima Cum Laude</i> National Latin Exam
1997	<i>Cum Laude</i> National Latin Exam

RESEARCH SUPPORT

EPA RD-83479801-0 (Consultant) Harvard School of Public Health, Clean Air Research Center	01/01/11-12/31/15 \$1,487,597
University of Louisville Sponsored Research Tuition Award Department of Physiology and Biophysics	01/02/11-05/14/11
NIH T35 ES-14559 Summer Research Scholar, University of Louisville	07/01/08-08/08/08 \$3,800
Summer Research Scholar, University of Louisville	07/01/07-08/10/07 \$3,000

BOOK CHAPTERS

1. **Bartoli CR**, Anderson M, Dowling RD. The Total Artificial Heart: Bridge-To-Transplant and Destination Therapy for End-Stage Biventricular Heart Failure. *Cardiothoracic Surgery Review*. 1st Edition. Lippincott Williams & Wilkins. 2011.

PEER REVIEWED PUBLICATIONS

1. **Bartoli CR**, Brittian KR, Giridharan GA, Koenig SC, Prabhu SD. Bovine model of doxorubicin-induced chronic heart failure. *Journal of Biomedicine and Biotechnology*. 2011, 2011:758736.
2. **Bartoli CR**, Godleski JJ, Verrier RL. Mechanisms mediating adverse effects of air pollution on cardiovascular hemodynamic function and vulnerability to cardiac arrhythmias. *Air Quality, Atmosphere, and Health*. 2011, 4:53-63.
3. **Bartoli CR**, Dowling RD, Wilson GC, Giridharan GA, Slaughter MS, Sherwood LC Spence PA, Prabhu SD, Koenig SC. A novel subcutaneous counterpulsation device: Acute hemodynamic responses to pharmacologically-induced hypertension, hypotension, and heart failure. Response to Letter to the Editor. *Artificial Organs*. 2011, 35(1):93-5.
4. **Bartoli CR**, Nadar MM, Godleski JJ. Capsule thickness correlates with vascular density and blood flow within foreign-body capsules surrounding surgically implanted subcutaneous devices. *Artificial Organs*. 2010, 34(10):857-861.
5. Nadar MM, **Bartoli CR**, Kasdan ML. Lipomas of the hand: a review and 13 patient case series. *ePlasty*, 2010, 10:e66.
6. **Bartoli CR**, Wilson GC, Giridharan GA, Slaughter MS, Sherwood LC Spence PA, Prabhu SD, Koenig SC. A novel subcutaneous counterpulsation device: Acute hemodynamic responses to pharmacologically-induced hypertension, hypotension, and heart failure. *Artificial Organs*. 2010, 34(7):537-45.
7. **Bartoli CR**, Giridharan GA, Litwak KN, Sobieski M, Prabhu SD, Slaughter MS, Koenig SC. Hemodynamic responses to continuous versus pulsatile unloading of the failing left ventricle. *ASAIO J*. 2010, 56(5):410-6.
8. Slaughter MS, Ising MS, Tamez D, O'Driscoll G, Voskoboynikov N, **Bartoli CR**, Koenig SC, Giridharan GA. Increase in circadian variation of flow after continuous flow ventricular assist device

- implantation. *Journal of Heart and Lung Transplant*. 2010, 29(6):695-7.
9. **Bartoli CR**, Spence PA, Giridharan GA. Novel J-stents reduce the risk of embolic stroke *in vitro*. *Neuroradiology*. 2010, 52(5):345-7.
10. **Bartoli CR**, Godleski JJ. Blood flow in the foreign-body capsule surrounding surgically implanted subcutaneous devices. *Journal of Surgical Research*. 2010, 158(1): 147-154.
11. **Bartoli CR**, Wellenius GA, Coull BA, Diaz EA, Lawrence J, Akiyama I, Okabe K, Verrier RL, Godleski JJ. Concentrated ambient particles alter myocardial blood flow during acute ischemia in conscious canines. *Environmental Health Perspectives*. 2009, 117(3): 333-337.
12. **Bartoli CR**, Wellenius GA, Diaz EA, Lawrence J, Coull BA, Akiyama I, Lee LM, Katz T, Okabe K, Verrier RL, Godleski JJ. Acute mechanisms of particulate air pollution-induced arterial blood pressure changes. *Environmental Health Perspectives*. 2009, 117(3): 361-366.
13. Slaughter MS, **Bartoli CR**, Sobieski MA, Pantalos GM, Giridharan GA, Dowling RD, Prabhu SD, Farrar DJ, Koenig SC. Intraoperative evaluation of the HeartMate II flow estimator. *Journal of Heart and Lung Transplant*. 2009, 28(1):39-43.
14. **Bartoli CR**, Okabe K, Akiyama I, Godleski JJ. Repeat microsphere delivery for serial measurement of regional blood perfusion in the chronically instrumented, conscious canine. *Journal of Surgical Research*. 2008, 145(1):135-41.
15. **Bartoli CR**, Akiyama I, Okabe K, Diaz EA, Godleski JJ. Permanent tracheostomy for long-term respiratory studies. *Journal of Surgical Research*. 2008, 145(1):124-9.
16. Gelzer AR, Koller ML, Otani NF, Fox JJ, Enyeart MW, Hooker GJ, Riccio ML, **Bartoli CR**, Gilmour RF. Dynamic mechanism for initiation of ventricular fibrillation in vivo. *Circulation*. 2008, 9;118(11):1123-9.
17. Kumar K, Nearing BD, **Bartoli CR**, Kwaku KF, Belardinelli L, Verrier RL. Effect of Ranolazine on ventricular vulnerability and defibrillation threshold in the intact porcine heart. *Journal of Cardiovascular Electrophysiology*. 2008, 19(10):1073-9.
18. **Bartoli CR**, Akiyama I, Godleski JJ, Verrier RL. Long-term pericardial catheterization is associated with minimum foreign body response. *Catheterization and Cardiovascular Interventions*. 2007, 70(2):221-227.
19. **Bartoli CR**, Okabe K, Akiyama I, Verrier RL, Godleski JJ. Technique for implantation of chronic indwelling aortic access catheters. *Journal of Investigative Surgery*. 2006, 19(6): 397-405.
20. **Bartoli CR**, Nadar MM, Kasdan ML. An atypical case of anesthesia-induced reverse Takotsubo cardiomyopathy in a 30-year-old male

- with post-traumatic stress disorder. *Journal of Cardiothoracic and Vascular Anesthesia*, In Press.
21. **Bartoli CR**, Dowling RD. The future of adult cardiac assist devices: Novel systems and mechanical circulatory support strategies. *Cardiology Clinics*, In Press.

PRESENTED ABSTRACTS

(* Award Winner, † Oral Presentation)

1. † Giridharan GA, **Bartoli CR**, Spence PA, Koenig SC, Dowling RD. Retrograde cerebral, aortic, and myocardial blood flow during IABP support. Proceedings of The American Society for Artificial Internal Organs, Washington, D.C., June 2011.
2. * Luckett AJ, **Bartoli CR**, Brittan K, Koenig SC, Spence PA, Dowling RD, D'Souza SE. A novel method for quantifying bovine von Willebrand factor and its cleaving protease, ADAMTS-13: Implications for acquired von Willebrand disease in LVAD patients. Proceedings of Research! Louisville, Louisville, KY, October 2010.
3. * **Bartoli CR**, Spence PA, Giridharan GA. Novel J-stents reduce the risk of embolic stroke *in vitro*. Proceedings of Research! Louisville, Louisville, KY, October 2009.
4. Slaughter MS, Ising MS, Tamez D, O'Driscoll G, Voskoboynikov N, **Bartoli CR**, Koenig SC, Giridharan GA. Reappearance of a normal circadian rhythm after ventricular assist device implantation. *Journal of Cardiac Failure*. 2009, 15(6): S51.
5. * **Bartoli CR**, Wilson GC, Giridharan GA, Slaughter MS, Sobieski M, Prabhu SD, Spence PA, Koenig SC. Testing of a subcutaneous counterpulsation device for the treatment of heart failure over a physiological range of hemodynamic conditions. Proceedings of Research! Louisville, Louisville, KY, October 2008.
6. Nadar MM, **Bartoli CR**, Greenberg RB. Medical school biostatistics and epidemiology curriculum analysis: suggestions for improvement at the University of Louisville. Proceedings of Research! Louisville, Louisville, KY, October 2008.
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PROFESSIONAL SOCIETIES

2005-present Academy of Surgical Research
 2007-present American Medical Association
 2007-present American Medical Student Association
 2008-present American Society for Artificial Internal Organs

PEER REVIEWER

2007-present Catheterization and Cardiovascular Interventions
2011-present American Society for Artificial Internal Organs
2011-present Archives of Toxicology

BIOMEDICAL CONSULTING

2008-present SCR Inc.
2009-present Hemoshield (Co-Inventor)
2009-present Telescopic Electrocautery Knife (Co-Inventor)