INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®
ALTERATIONS IN CANINE SUBMAXIMAL EXERCISE VO$_2$-KINETICS AND NEUROHORMONES DURING THE EVOLUTION OF HEART FAILURE PRODUCED BY RAPID VENTRICULAR PACING

DISSertation

Presented in Partial Fulfillment of the Requirements for

The Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Brian Michael Roche, B.S., M.Ed.

*****

The Ohio State University

2000

Dissertation Committee:

Robert Hamlin, Adviser

Timothy Kirby

S. M. Strauch

Kenneth McKeever

Approved By

Adviser

Department of Veterinary Biosciences
ABSTRACT

Reduced exercise capacity is a well-known concomitant of heart failure. Rapid ventricular pacing is an often-used method for producing heart failure in dogs. Producing heart failure by rapid ventricular pacing has become the method of choice for studying pathophysiology and therapy of heart failure. Although many hemodynamic and neuroendocrine parameters have been used to quantify the severity (degree) of heart failure, many health care professionals consider exercise capacity as a function that correlates strongly with severity of compromise of ventricular function characteristic of heart failure. There have been no studies of O_2 consumption, during exercise, in dogs with heart failure produced by rapid ventricular pacing. It is thought that among the parameters of exercise capacity, maximal oxygen consumption, maximal CO_2 production and respiratory exchange ratio (CO_2:O_2) at maximal exertion are the most important; but recently investigators have suggested that the rate of achieving a new plateau in oxygen consumption while performing 4 minutes of submaximal, constant treadmill activity is a simple and reproducible estimate of aerobic capacity.

The hypothesis of this study was that increasing severity of heart failure, produced by rapid ventricular pacing, results in an increase in time required for oxygen consumption to reach a new steady state. This study was also conducted to determine values of neurohormones during progressive increases in rapid ventricular pacing.
Mature, male hound dogs were instrumented with a programmable pacemaker. The dogs were then exercised at 3.7 mph 8% grade for 4 continuous minutes. Expired gas samples were collected every 10 seconds. Every three weeks the pacemaker's rate was increased (180, 200 and 220 bpm) and the dogs were re-exercised. Twenty-eight milliliters of blood was drawn at baseline and for each level of pacing. Atrial natriuretic peptide, aldosterone, anti-diuretic hormone, plasma rennin activity, norepinephrine, epinephrine and dopamine were analyzed.

Shortening fraction decreased with each level of pacing. Oxygen consumption during the fourth minute of constant work was decreased at 180 and 200 bpm compared to controls. The time to intersection point was not different at any level of pacing, showing a trend at 220 bpm. ANP and norepinephrine increased with each level of ventricular pacing. Dopamine increased with each level of pacing, becoming significant at 200 bpm.

The rapid ventricular pacing model employed in this study (increasing rates of 180, 200 and 220) induces an evolution of heart failure in dogs demonstrated by shortening fraction. The time to intersection point (VO\textsubscript{2} kinetics) increased with each pacing level.
Dedicated to my mother
ACKNOWLEDGMENTS

It is with utmost sincerity that I thank my advisor, Dr. Robert Hamlin, for believing in my abilities to successfully complete this degree. His knowledge, professionalism and kind-heartedness have led a fine example for me to follow.

My thanks to Dr. Tim Kirby for giving me the chance to pursue my degree, and the guidance he provided along the way. When I needed sound advice in the classroom or on a project Dr. Kirby was the one to go see.

I would like to thank Dr. S. Mark Strauch and Dr. Ken McKeever, without much needed and considerate effort on their part, this investigation would not have been realized.

A special thanks goes to Dr. Robert Lehnhard, it was his mentoring and leadership quality that has shaped me into the person that I am today. Words will not capture what his work ethic, knowledge and friendship has done to influence decisions that I have made in my professional and personal life. The sacrifices he has made and support he has shown cannot be repaid in one lifetime, and for that I am forever grateful.

And to my parents, for being patient and supportive over the years while I was attempting to build a solid educational base for what hopes to be a productive and flourishing life.
VITA

July 26, 1969............................... Born – Quincy, Massachusetts

1991............................................. B.S., The University of Maine, Orono, Maine

1992............................................. M.Ed., The University of Maine, Orono, Maine

1995-1998................................... Graduate Teaching Associate, School of Physical Activity and Educational Services, The Ohio State University, Columbus, Ohio

1998-2000................................... Graduate Research Associate, Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio

FIELDS OF STUDY

Major Field:  Veterinary Biosciences
             Exercise Physiology
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>v</td>
</tr>
<tr>
<td>Vita</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of Figures</td>
<td>x</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2. Review of Literature</td>
<td>4</td>
</tr>
<tr>
<td>2.1 Heart Failure and Functional Classification</td>
<td>4</td>
</tr>
<tr>
<td>2.2 Response to Exercise in Heart Failure Patients</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Models of Heart Failure in Dogs</td>
<td>9</td>
</tr>
<tr>
<td>2.3.1 Pressure and Volume loading</td>
<td>9</td>
</tr>
<tr>
<td>2.3.2 Cardiotoxic agents (Adriamycin)</td>
<td>11</td>
</tr>
<tr>
<td>2.3.4 Coronary Occlusion</td>
<td>12</td>
</tr>
<tr>
<td>2.3.5 DC Shocks</td>
<td>12</td>
</tr>
<tr>
<td>2.3.6 Tachycardia-induced Heart Failure</td>
<td>13</td>
</tr>
<tr>
<td>2.3.6.1 Left Ventricular Function</td>
<td>14</td>
</tr>
<tr>
<td>2.3.6.2 Hormonal Adaptations</td>
<td>15</td>
</tr>
<tr>
<td>2.4 Exercise and Heart Failure in Dogs</td>
<td>18</td>
</tr>
<tr>
<td>3. &quot;Alterations in canine submaximal exercise VO₂-kinetics during the evolution of heart failure produced by rapid ventricular pacing&quot;</td>
<td>22</td>
</tr>
</tbody>
</table>

vii
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>22</td>
</tr>
<tr>
<td>3.2</td>
<td>Materials and Methods</td>
<td>23</td>
</tr>
<tr>
<td>3.3</td>
<td>Data Analysis</td>
<td>25</td>
</tr>
<tr>
<td>3.4</td>
<td>Results</td>
<td>29</td>
</tr>
<tr>
<td>3.5</td>
<td>Discussion</td>
<td>35</td>
</tr>
<tr>
<td>3.6</td>
<td>Conclusions</td>
<td>38</td>
</tr>
<tr>
<td>3.7</td>
<td>References</td>
<td>39</td>
</tr>
</tbody>
</table>

**4.** "Changes in canine neurohormone levels with incremental increases in rapid ventricular pacing" ........................... 41

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Introduction</td>
<td>41</td>
</tr>
<tr>
<td>4.2</td>
<td>Materials and Methods</td>
<td>42</td>
</tr>
<tr>
<td>4.3</td>
<td>Data Analysis</td>
<td>45</td>
</tr>
<tr>
<td>4.4</td>
<td>Results</td>
<td>45</td>
</tr>
<tr>
<td>4.5</td>
<td>Discussion</td>
<td>54</td>
</tr>
<tr>
<td>4.6</td>
<td>Conclusions</td>
<td>57</td>
</tr>
<tr>
<td>4.7</td>
<td>References</td>
<td>57</td>
</tr>
</tbody>
</table>

5. SUMMARY ........................................................................................ 60

List of References ........................................................................................ 61
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Characteristics of Dogs During the Evolution of Heart Failure</td>
<td>30</td>
</tr>
<tr>
<td>4.1</td>
<td>Characteristics of Neuroendocrines and Catecholamines During Development of Heart Failure</td>
<td>48</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

“Alterations in canine submaximal exercise VO₂-kinetics during the evolution of heart failure produced by rapid ventricular pacing”

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>VO₂ curve</td>
</tr>
<tr>
<td>3.2</td>
<td>Calculation of Average VO₂ (Horizontal Line I)</td>
</tr>
<tr>
<td>3.3</td>
<td>Re-Averaged VO₂ (Horizontal Line II)</td>
</tr>
<tr>
<td>3.4</td>
<td>Calculation of Time to Intersection Point (TIP)</td>
</tr>
<tr>
<td>3.5</td>
<td>Changes in Shortening Fraction at Different Pacing Rates</td>
</tr>
<tr>
<td>3.6</td>
<td>Resting VO₂</td>
</tr>
<tr>
<td>3.7</td>
<td>Peak VO₂ after 4-minutes at a Constant Workload</td>
</tr>
<tr>
<td>3.8</td>
<td>Time (Seconds) to Intersection (TIP)</td>
</tr>
</tbody>
</table>

“Changes in canine neurohormone levels with incremental increases in rapid ventricular pacing”

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Changes in Shortening Fraction at Different Pacing Rates</td>
</tr>
<tr>
<td>4.2</td>
<td>ANP Values Following Increases in Ventricular Pacing Rates</td>
</tr>
<tr>
<td>4.3</td>
<td>ADH Levels Following Increases in Ventricular Pacing Rates</td>
</tr>
<tr>
<td>4.4</td>
<td>PRA Levels Following Increases in Ventricular Pacing Rates</td>
</tr>
</tbody>
</table>
4.5 Aldosterone Levels Following Increases in Ventricular Pacing Rates ........................................................ 51

4.6 Norepinephrine Levels Following Increases in Ventricular Pacing Rates ....................................................... 52

4.7 Epinephrine Levels Following Increases in Ventricular Pacing Rates ............................................................ 53

4.8 Dopamine Levels Following Increases in Ventricular Pacing Rates .............................................................. 54
CHAPTER 1

INTRODUCTION

In the United States heart failure has become a major health problem in the last 10 years. It is estimated that about four million Americans have heart failure with an estimated 465,000 new cases occurring each year. (Massie, B. M. & M. Packer, 1990) An estimation of 1 million hospitalizations annually with costs ranging from 15 to 30 billion dollars is attributed to heart failure. (Bello et al., 1999; Massie & Shah, 1997; Graves, 1995) As the American population ages, and the medical treatment for heart conditions continues to improve, the staggering costs of heart failure are going to continue to rise. The hope is that research will uncover markers (i.e., proteins, endocrines, VO$_2$) that will allow for early detection of heart failure. McAlister et al (1999) reports that more than 40% of patients with heart failure require readmission within 3 to 6 months of hospital discharge. Detecting the disease in its earliest form (class I NYHA) may decrease hospitalization visits and duration of stays. Primary prevention of heart disease (i.e., treatment of hypertension, lowering of cholesterol) has lead to reductions in the risk of developing heart failure. (O'Connell & Bristow, 1994; Kostis et al., 1997; Ghali, 1999) However, earlier detection of heart failure may lead to the administration of secondary preventive measures (i.e., angiotensin converting enzyme inhibitors, β-Blockers) which may also delay the development of the disease. (Ghali,
Experimental models of heart failure have been used to establish the pathophysiology and mechanisms of the disease. Of the models employed, rapid ventricular pacing produces the most predictable, stable condition of heart failure in dogs. In the past, models based on volume and pressure overload have been problematic due to difficulty in controlling the overload. (Smith & Nuttall, 1985) The techniques of venous occlusion (obstruction) and toxin administration also have control problems. (Smith & Nuttall, 1985) The extent of the myocardial damage induced by these methods is difficult to regulate and replicate. (Einstin & Abdul-Hussein, 1985) With the rapid-pacing model Spinale et al (1995) has shown disturbances to left ventricular function similar to those found in patients with cardiomyopathic disease. Furthermore, Seymour et al (1994) determined the response to rapid-ventricular pacing resembling the cardiovascular, renal and hormonal changes found in the clinical syndrome of heart failure.

The overall goals of these studies were to determine if submaximal VO₂-kinetics and endocrine responses are affected by the rate and duration of ventricular pacing. The specific objectives of the research were to determine if submaximal exercise performance relates to the severity of heart failure and if there are endocrine responses that parallel the degree of cardiac impairment. The hypotheses of the research were: (1) exercise performance defined by submaximal VO₂ remains unchanged at a given workload with increasing severity of heart failure, (2) VO₂-kinetics, defined by time to steady state, will increase with increasing severity of heart failure, (3) endocrine responses correlate to the severity of heart failure, (4) endocrine responses produced by rapid ventricular pacing will predict both the impairment of exercise performance and the degree of heart failure.
Severity of heart failure was assessed by percent decrease in left ventricular shortening fraction as measured by 2D-directed M-mode echocardiography.
CHAPTER 2

REVIEW OF LITERATURE

Heart failure and functional classification

Heart failure is a syndrome (i.e. a set of signs and symptoms) occurring when the cardiovascular system is unable to meet the metabolic demands of the body. Heart failure generally results from a pathological interaction between the heart and the peripheral circulation. It is characterized by changed hemodynamic, renal, neural, and hormonal profiles. The essential difference between the causes of the hemodynamic-neuroendocrine responses of heart failure versus shock is that in shock, there is inadequate venous return, while in heart failure; venous return is not the cardiac output-limiting phenomenon. A mandatory physiological occurrence in the clinical state of heart failure, is a failing heart. This is described in a laboratory of muscle physiology as a heart which has a reduced capacity to release energy from hydrolysis of ATP, which generates a reduced rate of shortening at zero load, and which generates less force than normal from a given end-diastolic volume. (Poole-Wilson, 1985) There are many variations on the definition of heart failure and most physiology books offer up their own. One consistency, however, is that there is ventricular dysfunction resulting in symptoms. At rest, the patient may appear asymptomatic unless in overt heart failure. The symptoms
of heart failure may become more expressed during exercise. (Lipkin et al., 1986) Symptoms may include breathlessness, fatigue, and fluid retention. Currently there is no single clinical measure that assures the identification of heart failure in individuals with heart disease. (Poole-Wilson et al., 1997) Because there is no gold standard in assessing heart failure there is great variability in its diagnosis.

There have been many published studies that have led to a scoring system in which heart failure patients are classified. Combining certain symptoms, physical examination and investigative findings, a score is achieved and the patient is classified as to the severity of heart failure. (McKee et al., 1971; Remes et al., 1992; Eriksson et al., 1989) Weber, Kinasewitz, Janicki, and Fishman (1982) and The New York Heart Association (NYHA) have established classifications that focus on symptoms, specifically exercise intolerance, that are important indicators of overall morbidity and mortality. Limitation of physical activity is the earliest symptom of heart failure (Jennings, & Esler, 1990) and the NYHA uses a subjective scale of shortness of breath (dyspnea) with exertion to identify heart failure. Weber et al., (1982) have developed a functional classification dependant upon maximum oxygen consumption measured during a graded exercise test. Risk classification is essential to identify the patients at the highest risk of mortality so that therapy can be instituted. Strategies for therapy (pharmacological, devices and transplantation) are employed according to such classification. (Poole-Wilson et al., 1997)
Response to exercise in heart failure patients

The normal response of the body to aerobic exercise is an increase in oxygen consumption (VO2). This is the amount of oxygen (ml/kg/min) which is consumed by the body's tissues. As the work being performed is increased, VO2 increases. Initially, VO2 is increased by cardioacceleration (up to 4 times resting) and to a lesser degree by augmentation of stroke volume (up to 25%) in humans. As exercise intensity continues to increase the remaining increases in VO2 are achieved by the increases in a-v O2 difference (O2 extraction). (Rowell, 1993) Functional capacity therefore is defined by the maximal amount of oxygen the body is able to consume during work (VO2 max). The Fick principle defines VO2 max as the product of maximal cardiac output (CO) = (Stroke Volume X Heart Rate) and maximal arteriovenous oxygen difference (a-v O2 difference).

The primary hemodynamic change during aerobic exercise is vasodilatation in working skeletal muscle. This occurs because working muscle produces metabolites, which relax vascular smooth muscle overcoming the higher adrenergic drive, which would result in its contraction. The vasodilatation and consequent increase in skeletal muscle blood flow helps meet the metabolic demands of increasing work. (Jennings & Esler, 1990) Along with an increase in the delivery of oxygen (CO), there is also an increase in the extraction of oxygen (a-v O2) by the exercising muscles. Skeletal muscle has the ability to vasodialate to a capacity where it can never be a limiting factor to VO2 in normal subjects. (Rowell, 1986) It is maximal CO therefore which limits VO2 max. (Rowell, 1993)

To permit the skeletal muscles to drastically increase their blood flow without a fall in blood pressure, blood flow to non-working tissues (e.g. splanchnic bed, kidneys) is
decreased by sympathetic induced vasoconstriction. As exercise intensity increases, vasoconstriction also increases. Along with venoconstriction, this results in an increased venous return. This augmented venous return plus the high adrenergic and low parasympathetic drive result in the increased in CO observed during exercise. Maximal CO is limited by maximal achievable heart rate.

In heart failure, there are cardiac and peripheral mechanisms, which partially compensate for the decrease in performance of the ailing left ventricle. (Jennings, 1990) Specifically, the left ventricle dilates (invoking the Frank-Starling mechanism), and the ventricle hypertrophies resulting in normalization of wall tension. (Gaasch, 1984) The decrease in systemic blood flow and decreasing arterial pressure with heart failure triggers the activation of neurohumoral mechanisms, which tends to normalize tissue perfusion. Increase in total peripheral resistance is provided by adrenergic stimulation, angiotensin II (AII), aldosterone and anti-diuretic hormone (ADH) or vasopressin. The increase in total peripheral resistance also increases afterload (the effort expended by the LV to eject blood into and through the arterial tree). This increased afterload results in a decreased stroke volume.

The decrease in cardiac output to the kidneys due to vasoconstrictor tone brings about salt and water retention. The increase in salt and water retention occurs even though the compensatory mechanisms for fluid retention are absent. Resulting in an increase in blood volume which helps to preserve blood pressure but which may result in hyponatremia and peripheral edema.

During exercise the heart failure patient is unable to meet the demands of the working skeletal muscles for delivery of oxygen. Impedance to the flow of blood is
increased due to increases in aortic impedance and total peripheral resistance. Skeletal muscle adapts to the decrease in cardiac output by decreasing is oxidative capacity. The left ventricle is unable to respond to the increase in venous return with exercise and dilatates. The increased venous return increases end diastolic pressure. Eventually, the increase in left ventricular filling pressure leads to increased pulmonary venous and capillary wedge pressures and in pulmonary congestion and edema.

Incremental exercise testing has been used to estimate the severity of heart failure in humans. Problems arise in the measurement of VO$_2$max within cardiovascular diseased patients due to subject’s motivation, as well as, the criteria used by technicians to terminate a max test. (Koike et al., 1995) The same problems identified in humans exist in the cardiovascular diseased dog. Maximal exercise testing maybe of limited value because the dog is presented to the clinic already in identifiable class IV heart failure and/or the maximal test is difficult to administer and dangerous to the dog. (Kittleson et al., 1996)

Recently, in humans, Koike et al (1995) showed that the time constant of VO$_2$ at the onset of a constant work rate exercise test is negatively correlated with peak VO$_2$ and maximal work rate. Belardinelli et al performed a follow up study to Koike, in 1998. Eighty patients with congestive heart failure performed a symptom limited ramping exercise test on a cycle ergometer and a submaximal constant work rate exercise protocol in random order on different days. The time constant of VO$_2$ was significantly longer for patients with CHF than in healthy subjects. The more severe the cardiovascular dysfunction, identified by peak VO$_2$, the longer the kinetics of VO$_2$, the shorter the time for respiratory exchange ratio to reach 1.0, and the greater value of the respiratory
exchange ratio at peak exercise. Multivariate analysis revealed that the time constant of VO₂ and the time to reach a respiratory exchange ratio of 1.0 are the best predictors of cardiovascular functional class.

**Models of Heart Failure in Dogs**

The mechanisms involved in heart failure are difficult to investigate in humans because of ethical limitations and the fact that most patients present late in the disease process. To study the mechanisms involved in heart failure in man a stable animal model is needed. The animal model of heart failure should mimic human heart failure and allow for the study interventions. The degree of dysfunction should be predictable, quantifiable and controllable and should satisfy economical, technical and animal welfare considerations. (Einstin & Abdul-Hussein, 1995)

**Pressure and Volume Loading**

Heart failure may be produced by exposing (i.e. perturbing) the heart to pressure and/or volume overloading. Typical pressure overload is produced by aortic or pulmonic stenosis, or possibly by systemic arterial hypertension. These perturbations lead to concentric hypertrophy, in which the ventricular lumen is relatively small, compared to the thick ventricular walls. Volume overload may be produced by creating a left to right intra (e.g. ASD, VSD) or extra cardiac (e.g. PDA, a-v fistula) shunt, either atrioventricular or semilunar valvular insufficiency or rapid pacing. These perturbations
lead to eccentric hypertrophy, in which the ventricular lumen is relatively large, compared to the relatively thin ventricular walls.

Ventricular afterloads of volume and pressure overload differ dramatically. Afterload is estimated as the product of lumen radius and intraventricular pressure divided by ventricular wall thickness. Pressure and thickness are measured at the instant the aortic valve (or pulmonic valve) opens. With pressure overload, because the lumen radius is small and the wall is thick, the myocardium generates much less tension than with volume overload, in which the radius is large and the wall is thin. Thus myocardial oxygen consumption is much higher with volume overload and myocardial destruction due to mismatch between oxygen consumption and delivery is greater. This makes the patient with a volume overload more susceptible to developing heart failure.

Pressure overload may be produced by renal artery occlusion, as well as, using constrictive bands on major outflow tracts—usually the aorta. These techniques induce systemic hypertensive, pressure overloading of the heart. (Smith & Nuttall, 1985) Pressure overload heart failure is similar to some instances of heart failure occurring in humans except for the fixed, acute afterload which differs from the more gradual overload that occurs with humans. (Einstin & Abdul-Hussein, 1995) For this reason, Crozatier, Caillet, and Bical in 1984 suggested that the model of pressure overload may not be suitable for the evaluation of new drugs. The difficulty with volume overload is the ability to determine the time to onset and severity of heart failure after the surgical damage to the aortic valve has occurred. (Einstin & Abdul-Hussein, 1995)
Cardiotoxic agents (Adriamycin)

This antineoplastic compound is thought to produce myocardial damage by the production of free radicals of oxygen. At doses, in dogs, greater than 180 mg/M2 almost all dogs will develop reduction in ventricular function often leading to heart failure and occasionally to sudden death. The compound is slightly less toxic to humans, but at high doses produces heart failure in humans as well. The difficulty with adriamycin (doxorubicin) as a model for heart failure lies in the lack of uniformity of the heart failure developed. Other problems include the rather long time between initiation of therapy and development of compromised ventricular function, and the fact that doxorubicin also immunocompromises leading to infection. The yield of good models for chronic heart failure with this method is not high, and is very expensive. However, there is greater reproducibility and less cost if the compound is injected directly into the coronary circulation (7.5 mg/kg every week for 5 weeks).

The administration of doxorubicin produces cardiomyopathy with pathological, hemodynamic and hormonal changes similar to those observed in humans with heart failure, but the dose to produce sufficient but not excessive damage to the heart is difficult to pin point. (Einstin, 1995; Toyoda, 1998; Tomlinson, 1985) This model has also reported that administration of adriamycin into the left main coronary artery causes left ventricular dysfunction without compromising right ventricular function. (Magovern et al., 1992)
Coronary Occlusion

Heart failure may be induced acutely by complete obstruction of a major coronary artery, by slowly obstructing a major coronary artery, or by progressively obstructing small coronary arterial branches. Obstruction may be by ligation, injection of microspheres, or even by thromboembolization. Problems with this model include mortality due to arrhythmias, the growth of collateral vessels that prevents the onset of heart failure, and the lack of uniformity of heart failure. (Smith & Nuttall, 1985).

DC Shock

Heart failure has been shown to occur after repeated DC shocks across the left ventricle. (McDonald et al, 1992) This is performed by applying DC energies, every 15 to 30 seconds, directly across the left ventricular free-wall. An electrode is placed within the left ventricle, and the second of the pair is placed on the thorax directly above the left ventricle. Up to 6 discharges of approximately 75 J are required. Following the shocks, the animal must recover for months in order for heart failure to develop. The model produces similarities to human heart failure in that there is a decrease in cardiac output and ejection fraction along with an increase in norepinephrine levels. A major difference with DC shock therapy and human heart failure is the absence of an increase in plasma renin activity. (Einstin & Abdul-Hussein, 1995) The limitation of this method, as well as coronary occlusion, is that the disease does not mimic cardiomyopathy, but is more similar to acute myocardial infarction.
Tachycardia-induced heart failure

Human patients who developed congestive heart failure (CHF) from chronic supraventricular tachycardia, led investigators to believe that an artificially paced model could be developed to create the same condition. The first report of a tachycardia-induced heart failure model was by Whipple and colleagues in 1962. These investigators modified an existing pacemaker to stimulate the atria of a dog at a rate of 330 beats per minute (BPM) (Whipple et al, 1962). This model induced heart failure, but also lead to atrioventricular (AV) node block.

Wilson, Douglas, Hickey, Lanoce, and Ferraro, 1987, and others, modified Whipple’s model by pacing the ventricles directly (Riegger, & Liebau, 1982; O’Brien et al, 1990; Travil et al, 1992; Seymour et al, 1994). They found that a rate of 250 ± 10 BPM produced signs and symptoms consistent with biventricular failure. These included: increased ventricular filling pressure, reduced ejection fraction, cardiac dilatation, and ascites. These investigators also found that with the cessation of pacing, and return to normal sinus rhythm, that ventricular function improved (Travil et al, 1992; Gallagher, 1985).

Studies incorporating the rapid pacing model in dogs uniformly use pacing rates of 250 ± 10 BPM over the course of 21 ± 7 days. This rate and time frame consistently elicit dyspnea, ascites, and a third heart sound as well as the following changes in hemodynamics and cardiac function: increased left ventricular end diastolic pressure (LVEDP), increased right atrial pressure (RAP), increased pulmonary capillary wedge pressure (PCWP), decreased ejection fraction (EF), decreased cardiac output (Q) and decreased mean arterial pressure (MAP). (Wilson et al, 1987; Seymour et al, 1994;
Sasayama et al, 1992) Changes in neurohumoral function in dogs due to this pacing model have been documented as well. These changes include increases in atrial natriuretic peptide (ANP), plasma renin activity (PRA), angiotensin II (AII), aldosterone, norepinephrine, epinephrine, and antidiuretic hormone (ADH) (Riegger, & Liebau, 1982; Spinale et al, 1995; Riegger et al, 1984).

Left ventricular function: Etiologies of human congestive heart failure (CHF) are unclear, the one consistent finding is the inability of the left ventricle to maintain cardiac output. Rapid ventricular pacing affects left ventricular function in a manner similar to human CHF. (Armstrong et al, 1986; Wilson et al, 1987; Komamura et al, 1993; Perreault et al, 1992) Studies have shown that progressive left ventricular dilation over a 3 to 4 week period and significant decline in LV ejection performance occur with chronic ventricular pacing. (Armstrong et al, 1986; Wilson et al, 1987; Komamura et al, 1993; Perreault et al, 1992; Spinale, 1996) Pacing the ventricle for periods upward of 6 weeks reduces LV ejection fraction and the peak rate of developed pressure (+dP/dt). Furthermore, there is an increase in LV end diastolic volume with a concomitant decrease in stroke volume. (Komamura et al, 1993)

Decreased LV wall thickness occurs with no significant change in LV mass with rapid ventricular pacing. (Armstrong et al, 1986; Wilson et al 1987) Consequently, there is an increase in LV peak systolic wall stress during the development of rapid pacing induced heart failure. (Spinale, 1996) Perreault, Shannon, Komamura, Vatner, and Morgan (1992) demonstrated in isolated pappilary muscle in dogs, with pacing induced heart failure, produced less tension in response to increasing Ca^{++} content than controls.
Abnormalities in myocardial relaxation, as demonstrated by increasing LV end diastolic pressure and decreasing LV filling rate have also been demonstrated. (Spinale et al, 1991)

Left Ventricular contractile responsiveness to β-adrenergic receptor stimulation has been shown to be impaired with rapid ventricular pacing. (Spinale, 1996) This downregulation of β-adrenergic receptor number is also seen in human heart failure. (Poole-Wilson et al, 1997)

Hormonal adaptations with rapid ventricular pacing: A number of studies have used the rapid pacing model to investigate the neuroendocrine response to heart failure in dogs. Stevens, Burnett, Kinoshita Matsuda and Redfield (1995) and others (Redfield et al. 1993; Riegger et al., 1988; Seymour et al., 1994; Moe et al., 1990; Shen et al., 1996; Perrella et al., 1992; Travill et al., 1992) have profiled changes in atrial natriuretic peptide (ANP) with various pacing rates and durations. Findings were similar in all of these studies. Plasma levels of ANP rise immediately and peak within the first five days of pacing, then plateau slightly below peak values for the remainder of the pacing period.

Riegger et al in 1988 was one of the first groups to assess plasma ANP using the rapid ventricular pacing model. Six dogs were paced at 260bpm for nine days. Plasma levels of ANP were measured on days 3, 6, and 9. ANP levels rose significantly (p>.03) after 3 days. The levels continued to rise and peaked by day 6. Plasma ANP slightly declined by day 9 but remained significantly higher than baseline. The author's concluded that an increase in ANP during the development of heart failure is related to an increase in right atrial pressure.
Increasing the pacing duration to 5 weeks, Moe et al (1990) studied 8 dogs paced at 250bpm until severe heart failure developed. After the first week of pacing plasma ANP levels increased from 98ng/L to the peak level of 422ng/L. The levels plateau at 354 and remained for the last 4 weeks of pacing. Moe states that plasma atrial natriuretic peptide plateaus in spite of the progressive increase in pulmonary capillary wedge pressure. Furthermore, the release of ANP becomes attenuated as severe heart failure develops.

Redfield et al (1993) and Stevens et al (1995) explored the use 180bpm for 10 days to investigate ANP and the antagonistic effect ANP has on the renin-angiotensin-aldosterone system (RAAS). They found that pacing the dogs at 180 beats per minute for 10 days resulted in a rise in ANP similar to that documented by previous researchers. They also compared the ANP values to the renin-angiotensin values and found that as ANP levels remained elevated the renin system did not change. They concluded that in the early stages of heart failure increased levels of circulating ANP may keep the vasopressive renin system from increasing.

The physiological function of the renin-angiotensin-aldosterone system (RAAS) system has been well documented in the literature in both humans and dogs. (Poole-Wilson et al., 1997) The RAAS system is a cascade of hormones that is activated under sympathetic response. During heart failure, the increase in sympathetic activity to the kidney increases the release of renin. Circulating levels of renin are cleaved by angiotensinogin in the liver to form angiotensin I (AI). The circulating level of AI is then cleaved by angiotensin converting enzyme in the lung to form the potent vasoconstrictor angiotensin II (AII). Both circulating renin and AII stimulate the adrenal cortex to form
and release aldosterone. Aldosterone affects the distal tubules of the kidney to reabsorb sodium and water.

Studies by Riegger et al. (1988) and others (Stevens et al., 1995, Spinale, 1996) have shown how the cascade of catecholamines is increased with the evolution of heart failure. In heart failure there is a four to five fold increase in circulating levels of norepinephrine. This increase stimulates the sympathetic nervous system, in an effort to produce a more forceful contraction, in order to maintain cardiac output. Increased levels of norepinephrine in turn increase the release of rennin, which increases AII. The heart is failing and the body, in an effort to maintain cardiac output, releases vasoconstrictive hormones that actually worsen the condition. The elevated ANP levels in the early stages of heart failure are the body's attempt to offset the fluid retention caused by AII and the failing heart. However, the ANP response is limited by its storage and release as shown by decreasing ANP levels with increasing heart failure. (Perrella et al., 1992)

As reported above, circulating levels of norepinephrine increase during heart failure. Other catecholamines, specifically epinephrine and dopamine, also increase. Spinale et al. (1996) investigated the response of norepinephrine, epinephrine and dopamine to 38 days of rapid pacing at 180, 200, 210, 220 and 240 beats per minute. Norepinephrine increased incrementally with each increase in rate. Epinephrine followed this same pattern with an exception at 210bpm, where they reported a slight decrease. Dopamine remained unchanged until the dogs were paced at 220bpm where the values doubled. The values doubled again when pacing was increased to 240bpm. (Spinale, 1996)
With heart failure, antidiuretic hormone (ADH) is released from the pituitary in response to increased angiotensin-II. The increased angiotensin-II results, possibly, from abnormal high-pressure baroreceptor function, resulting from loading of those baroreceptors with Na-K ATPase produced by high levels of circulating aldosterone. The increased aldosterone also occurs because of increased angiotensin-II. Circulating ADH acts to increase permeability of water by the distal tubules and collecting ducts of the kidney causing excessive resorption of water into the relatively hyperosmotic renal parenchyma and thence into the peritubular capillaries. (Poole-Wilson et al., 1997) To date however, there has been little research involving the role of this hormone in heart failure produced by rapid ventricular pacing.

**Exercise and Heart Failure in Dogs**

In the human clinical setting, patients are classified as to the severity of heart failure by exercise intolerance. To assess exercise intolerance or the severity of cardiovascular disease, incremental exercise testing has been used. More specifically, peak oxygen consumption (VO$_2$ max), a variable typically measured during a graded exercise test, positively correlates with cardiovascular function. (Weber, 1985 & Rowell, 1993) The pitfall of using an incremental exercise test to maximum effort is attaining reproducible data in heart failure patients. (Koike et al., 1995) Ideally, the criteria for termination of a graded exercise test should be subject fatigue or exhaustion. Often however, a test is terminated due to a lack of motivation on the part of the subject or because of symptom-limiting criteria employed by the attending physician. Continued research within the field of heart failure has focused on the causes of exercise intolerance.
during this state, more specifically decreases in cardiac output and inadequate perfusion of skeletal muscle. Currently, scientists are investigating models of exercise testing that will accurately identify central and peripheral shortcomings.

Rapid ventricular pacing in dogs is currently reported as the most stable and predictable model of heart failure for use in research. (Komamura et al., 1993; Moe et al., 1992; Riegger & Liebau, 1982) Interestingly, research to date is limited in exploring exercise performed with rapid ventricular pacing in the dog. (Hammond et al., 1997; Kittleson et al., 1996; Neumann and Heusch, 1997) Hammond et al. (1997 abstract) rapidly paced dogs at 225bpm for ~3 weeks to induce moderate heart failure. The dogs exercised at a moderate workload (3.2kph - 0% grade) and heavy workload (8kph - 15% grade). The authors recorded mean arterial pressure (MAP), cardiac output (Q), heart rate (HR), central venous pressure (CVP), and renal blood flow at rest and during moderate and heavy workloads. They found that for all data collection points (rest, moderate exercise and heavy exercise) MAP and Q decreased, and HR and CVP increased for heart failure dogs compared to control dogs. The abstract reports that measurements were taken during a steady state but fails to report the duration of submaximal exercise at the different workloads.

Kittleson et al (1996) used submaximal exercise to determine differences in lactate threshold in dogs serving as controls and dogs in class IV heart failure. The rapid ventricular model used by Kittleson (1996) was pacing at 260bpm for 2 – 3 weeks. The exercise workloads were 1, 2, and 3 miles per hour at a 16% grade, and 3 miles per hour at 22% and a 26% grade. The dogs exercised at the initial workload and blood lactate and blood gas measurements were taken at 5 and 15 minutes. The dogs were then
allowed to rest for 20 minutes. The dogs were then placed back on the treadmill and the exercise was increased to the next workload until 1.0 mmol/L blood lactate concentration was determined. All of the heart failure dogs achieved a 1.0 mmol/L lactate concentration at 5 minutes of the first exercise workload (1 mph 16%), while normal dogs achieved 1.0 mmol/L at various workloads; 3 at the 3 miles per hour at a 16% grade, 5 at 3 mph 22% grade and 4 at 3 mph 26% grade. Kittleson (1996) concluded that the exercise protocol can be used to distinguish dogs with class IV heart failure from normal dogs.

Neumann and Heusch in 1997 investigated exercising the control dogs and rapidly paced dogs on a treadmill at 5 km/g for 10 minutes. In addition to determining cardiac output at rest and during steady state exercise, the author’s also collected blood for lactate and catecholamine measurement. The results showed that the heart failure dogs had significantly decreased cardiac output at rest and during exercise compared to controls. Furthermore, the heart failure dogs demonstrated significant increases in Norepinephrine and Epinephrine at rest and during exercise compare to control dogs. The control dogs reached a level of 0.7 mmol/L of lactate during exercise, whereas, the heart failure dogs reached a level of 1.1 mmol/L. Thus, the conclusion from this study was that exercise intolerance is evident in the pacing-induced heart failure model.

Previous researchers have used a variety of pacing rates and time frames to produce heart failure in dogs. To date, there has not been the establishment of a standardized protocol. The broad purpose of this study was to develop a valid and reliable rapid pacing model in the hope of standardizing future research in this area.
More specific purposes included the characterization of heart failure through changes in 
VO₂ submaximal kinetics and various endocrine measures.
CHAPTER 3

ALTERATIONS IN CANINE SUBMAXIMAL EXERCISE VO$_2$-KINETICS DURING THE EVOLUTION OF HEART FAILURE PRODUCED BY RAPID VENTRICULAR PACING

Introduction

Reduced exercise capacity is a well-known concomitant of heart failure. Although many hemodynamic and neuroendocrine parameters have been used to quantify the severity (degree) of heart failure, many health care professionals consider exercise capacity a strong correlate to the severity of compromise of ventricular function characteristic of heart failure. Rapid ventricular pacing is an often-used method for producing heart failure in dogs. (Whipple et al 1962) Producing heart failure by rapid ventricular pacing has become the method of choice for studying pathophysiology and therapy of heart failure. However, there have been no studies of O$_2$ consumption, during exercise, in dogs with heart failure produced by rapid ventricular pacing. It is thought that among the parameters of exercise capacity, oxygen consumption, CO$_2$ production and respiratory exchange ratio (VCO$_2$/VO$_2$) at maximal exertion are the most important. Recently, however, investigators have suggested that the time to achieve a plateau in oxygen consumption while performing
4 minutes of submaximal, constant treadmill activity is a simple and reproducible estimate of aerobic capacity. (Kioke et al. 1995, Belardinelli et al. 1998) Kioke and Belardinelli used the time constant (tau) to quantify the rate of increase of oxygen consumption. Tau is the time required to proceed from resting VO$_2$ to 63% of the new steady state VO$_2$ achieved during 4 minutes of submaximal exercise.

The purpose of this study was to determine changes in O$_2$ consumption during a constant workload at varying rates of rapid ventricular pacing in dogs. It was the further purpose to determine what changes, if any, occur in the time to steady state with increasing severity of heart failure.

Materials and Methods

Subjects

Fourteen large (25-35 kg) mature, male, hound-type dogs were used in this study. Each dog was determined to be in good health by; appetite, general appearance and physical examination. The physical examination included auscultation of the heart and lungs, thoracic radiography, electrocardiography and echocardiography. The other primary inclusion criterion for this study was a willingness by the dog to run on a motorized treadmill while wearing a facemask.

Pacing Protocol

A magnetically programmable pacemaker was implanted, aseptically, to pace the right ventricle at various rates. (Redfield et al., 1993) Under anesthesia a small incision was made in the cervical region, and the pacing leads were threaded through the maxillary branch of the jugular vein, and impacted on the endocardium of the right
ventricle. The trabeculae carnea were used to hold the leads in place. The pacemaker was interred subcutaneously in a neck pouch, and the incision was sutured closed.

The pacing protocol consisted of progressive increases in heart rate at three-week intervals. Pacing began at 180bpm for the first three weeks, and was increased to 200bpm for weeks 4 through 6, and 220bpm for weeks 7 through 9.

**Exercise Protocol**

The dogs were placed on a stationary treadmill and connected to a shoulder harness. The variable rate pacemaker was inactivated (30bpm) prior to exercise. From standing rest, the dogs began exercise at a speed of 3.7 mph and a grade of 8 percent. This workload was maintained for a period of 4 minutes and then the treadmill was shut off. At the conclusion of exercise the dog’s pacemaker was re-activated.

**Measurement of VO\textsubscript{2}**

VO\textsubscript{2} was measured with the dogs standing on the treadmill at rest, and throughout the four-minute exercise session. The dog was fitted with a facemask designed for channeling expired air into an Oxymax open-flow system (Columbus Instruments, Columbus OH). This system consisted of a computer, thermister flow meter, a chemical O\textsubscript{2} sensor, and an infrared CO\textsubscript{2} sensor. Air was drawn through a filter by a vacuum pump at 400L/min and expired samples were analyzed for O\textsubscript{2} and CO\textsubscript{2} content every 10 seconds. The gas analysis produced values for VO\textsubscript{2}, VCO\textsubscript{2} and REQ at these same 10-second intervals. During collection of a resting VO\textsubscript{2} sample, the dog was verbally encouraged to remain calm. When the VO\textsubscript{2} reached a resting steady state (values remained within 1 ml/kg/min for 3 sampling points or 30 seconds), the treadmill was
activated and exercise began. Calibration of the oxygen consumption apparatus was completed prior to each exercise session.

**Shortening Fraction (ejection fraction):**

Ejection fraction (EF) is the time-honored method of assessing ventricular function. It is the ratio of stroke volume (SV) to end-diastolic volume (EDV) of the left ventricle. EF is calculated by subtracting end-systolic volume (ESV) from EDV to obtain SV, and then dividing SV by EDV. In this study, left ventricular shortening fraction (SF) was used as a surrogate for EF. SF was calculated by 2-D directed M-mode echocardiography, in which EDD (end-diastolic diameter) and ESD (end-systolic diameter) were averaged over 3 cardiac cycles. The cursor was placed just apically to the mitral apparatus. EDD was measured at the beginning of the QRS complex, and ESD was measured at the peak of the T wave on the ECG. Both FS and EF have been shown to correlate closely with one another and with the severity of compromise of left ventricular function in heart failure. (Poole, 1997; Fortun et al, 1971) Twelve hours prior to each exercise session, the dog’s pacemaker was inactivated and each dog received 0.8 mg/kg (body weight) of Butorphanol subcutaneously. Fifteen minutes later, echocardiography was performed to determine SF. At the end of the procedure the pacemaker was re-activated.

**Data Analysis**

The VO₂ for each 10-second interval during exercise was plotted. Each resulting oxygen consumption curve contained an initial steep rise, followed by a short decline, followed by a plateau. (Figure 3.1) Time to steady state during exercise was determined in the following manner. VO₂ was averaged over the entire 4-minute exercise bout. This
average value was drawn as a horizontal line (I) across the VO$_2$ curve (Figure 3.2). The oxygen consumption values preceding the intersection of horizontal line (I) with the steep portion of the curve were subtracted from the original total number of points, and the remaining number of points was then re-averaged. This re-averaged value was drawn as a horizontal line (II) across the VO$_2$ curve (Figure 3.3). The time from onset of exercise to the intersection of horizontal line (II) with the steep portion of the curve was identified as the "time to intersection point" (TIP). (Figure 3.4) The TIP value was used as the measure for VO$_2$ kinetics for each trial.

![VO2 Curve](image)

**Figure 3.1.** VO$_2$ Curve
Figure 3.2. Calculation of Average VO₂ (Horizontal Line I)

Figure 3.3. Re-Averaged VO₂ (Horizontal Line II)
Figure 3.4. Calculation of Time to Intersection Point (TIP)

The statistical analysis performed on the data was an analysis of variance with repeated measures. Mean values at baseline, 180bpm and 200bpm were compared using a sample of 14 subjects. Due to subject mortality the data presented for 220bpm had a sample size of six. The mean values for this treatment were plotted to help establish a trend line, but they were not included in the repeated measures. Bonferroni post-hoc tests were administered whenever the F-test indicated a $p<0.05$ for main effects.
Results

Not all of the fourteen dogs, which began this study, were able to complete the entire pacing protocol. Between the 6th and the 9th week period, when pacing had increased from 200bpm to 220bpm, eight dogs were removed from pacing and dropped from the study due to the severity of their symptoms. This left us with an n of 6 for the final treatment (220bpm).

Prior to the start of the study we had planned to treat the data with repeated measures analysis of variance (ANOVA). However, with the large loss in the number of subjects, it was decided to exclude the last treatment (220bpm) from the repeated measures analysis and report the findings from this group in a descriptive manner to help illustrate any possible trend lines in the data. Any significance determined with the ANOVA was treated further with a Bonferroni post-hoc analysis.

Body weight in the dogs dropped from 30.5 ± 1.03 kg to 28.3 ± 1.46 kg from baseline to the end of 220bpm pacing period (9 weeks) respectively. This loss in body weight was not significant (Table 3.1).
Mean shortening fraction (SF) decreased with each increase in pacing rate. At 180bpm there was a significant decrease from the baseline of 35.5 ± 1.36 % to 25.0 ± 1.42 % (p < .05). The SF of 19.5 ± 1.87 % measured at 200bpm was significantly different from 180bpm and baseline (p < .05). At 220bpm the mean SF of 12.2 ± 2.27 % continued the downward trend from 180bpm and 200bpm. Shortening fraction results are presented in Table 3.1 and Figure 3.5.
Values = mean ± SE
*Significantly different from baseline (p<.05)
**Significantly different from baseline and 180bpm (p<.05)

Figure 3.5. Changes in Shortening Fraction at Different Pacing Rates.

The only significant difference in mean resting VO₂ (ml/kg/min) was between 180bpm (12.7 ± 0.96) and baseline (17.7 ± 1.18) (p < 0.05) (Table 3.1, Fig. 3.6).
Figure 3.6. Resting VO₂

However, mean peak VO₂ (ml/kg/min) significantly decreased from baseline (47.5 ± 2.4) to 180bpm (42.0 ± 1.86) and again to 200bpm (36.6 ± 1.60) (p < .05). This downward trend continued at 220bpm with a mean value of 29.8 ± 3.57 (Table 3.1, Fig.3.7)
Values = mean ± SE
*Significantly different from baseline (p<.05)
**Significantly different from baseline and 180pkm (p<.05)

**Figure 3.7.** Peak VO₂ After 4-minutes at a Constant Workload
Mean values for the time to intersection (seconds) are presented in Table 3.1 and Fig. 3.8. Although there was an increasing trend in the time to intersection, none of the increases were significant.

Values = mean ± SE

**Figure 3.8.** Time (seconds) to Intersection (TIP)
Discussion

Previous researchers have used various pacing rates and durations to elicit heart failure in dogs. (Parrella et al., 1992; Moe et al., 1990; Redfield et al., 1993; Stevens et al., 1995; & Riegger et al., 1982) With the exception of Redfield (1993) and Stevens (1995), who employed 180bpm to investigate the early effects of heart failure, previous studies have used rates of 200bpm or greater with the intent of producing severe heart failure. The purpose of this study was to progress the dogs, in a step-wise fashion, through the evolution of heart failure (mild, moderate and severe) in order to investigate corresponding changes in submaximal VO₂-kinetics.

Shortening fraction (SF) was used as the criteria to mark the evolution of heart failure. (Burkett et al., 1994) The results indicate that we were successful in eliciting a graded approach to severe heart failure. Normal, resting SF for a dog is 35 % and our baseline data corresponded to this. Three weeks of pacing at 180bpm reduced SF to 25 % (a 40% reduction). Increasing the pacing rate to 200bpm for the next 3-weeks reduced SF even further to 19.5 %. The trend of decreasing SF with an increase in pacing rate continued when the rate went to 220bpm. After 3-weeks at this rate SF fell to 12 % indicating severe or overt heart failure. Our model clearly demonstrated an incremental increase in the severity of heart failure, from mild to moderate to severe with 3-week incremental increases in ventricular pacing rate.

Resting oxygen consumption in the normal dog is ~10 ml/kg/min. (Snow, 1985). Our resting values were higher than normal probably due to anticipatory responses in the dogs immediately prior to exercise. Anticipation of exercise was particularly high in the dogs during collection of our baseline data. They hadn’t as yet experienced the effects of
any pacing and were difficult to settle down due to their eagerness to walk on the treadmill.

$VO_2$ at 4 minutes ($VO_2$ peak) of exercise was defined as the average of the last 30 seconds (3 sample points) of exercising oxygen consumption. At the onset of the study we expected oxygen consumption to remain unchanged during the constant workload even with increasing pacing rates. What we found was a significant decrease in $VO_2$ peak with each increase in pacing rate. This decrease in $VO_2$ was not collaborated however, by an increase in respiratory exchange ratio (RER), which remained unchanged throughout the pacing increases. The decrease in $VO_2$ may be explained by the harness system worn by the dogs while on the treadmill. The chest harness worn while performing the treadmill exercise was connected to an overhead support. As long as the dogs exercised at the front of the treadmill the harness lent no support to the work being done. As the dogs progressed through the pacing protocol, and the severity of heart failure increased, their reliance on the harness for support also increased. The increasing support (of body weight) by the harness, decreased the amount of work being performed resulting in a decrease in oxygen consumption. This same phenomenon has been documented in human studies when subjects walking on a treadmill were allowed to hold on to handrails. (Astrand, 1982) Studies in horses have also demonstrated this effect when running on the treadmill and the lead rope is pulled from the front. (Unpublished, McKeever) Both are examples of decreases in $VO_2$ without a decrease in workload.

As reported in the data analysis, we suffered from subject “drop-out” as a result of the dogs reaching what we established as end point criteria— they would have died had pacing at the higher rate been continued. Our criteria for removing a dog from the study
consisted of a SF of <17% and/or physical signs (i.e., ascites, crackles, shadows on the radiograph, and lethargy). The 6 dogs that constituted the 220bpm group no doubt possessed certain properties that allowed them to survive at that pacing rate whereas the others (i.e. the “drop-outs”) would have died. Had the “drop-outs” been included in this group, no doubt the parameters that were measured would have been much more abnormal than those shown by the 6 dogs that were included. None-the-less parameters from even the 6 dogs that were included showed obvious clinical—albeit not statistical—differences from the dogs paced at 200bpm.

Our method for calculating the time required for attaining a steady state during exercise (TIP) was necessitated by two features of the VO$_2$ curve (Fig. 3.1). As is common in dogs (and horses), with the start of exercise, VO$_2$ “overshoots” the level actually required to sustain the given submaximal workload. This has been explained as an anticipatory phenomenon often demonstrated in these species prior to exercise. Alternately, this over-shoot may be caused by the increased energy demanded to overcome the inertial forces manifested at the beginning of motion, or by decreased efficiency (possibly economy) of the gate at the onset of activity. Also, our curves frequently showed a decrease in VO$_2$ during the final minute of exercise. As the severity of heart failure increased with each increase in pacing rate, it becomes more difficult for the dogs to maintain a steady state for 4-minutes. They kept pace with the treadmill (workload) by relying on the safety harness for support of their body weight. Thus, VO$_2$ was shown to decrease even though walking speed remained unchanged. The time to intersection point (TIP) was calculated, therefore, as described in our methods, for each
dog at each level of ventricular pacing. The intra-coefficient of variation for our calculation of (TIP) was 10.8%.

At the onset of exercise, the increase in VO$_2$ reflects the immediate increase in cardiac inotropy, increased venous return, and heart rate. (Wasserman, 1988) Weissman et al (1982) found that, in humans, the early dynamics of VO$_2$ at the onset of a constant workload exercise session correlates with the kinetics of early cardiac output increases and should be affected by cardiovascular functional class. Thus, the expectation for this study was for (TIP) to elongate (increase in time) with each level of ventricular pacing due to a slowing of VO$_2$ kinetics as the dogs progressed from mild to severe heart failure. We observed such a trend. This slowing of VO$_2$ kinetics is the result of a decrease in the delivery of oxygen by the heart (cardiac output) and/or a decrease in oxygen consumed by the working muscles (a – v O$_2$ difference). The most dramatic increase in (TIP) occurred at 220bpm. Had the subject number remained at 14 for that pacing period (220bpm) we believe that the difference of 57.6 seconds (220bpm) from 41.9 seconds (baseline) would have been significantly different.

In conclusion, this study documents that an increasing ventricular pacing rate over time elicits an evolution of heart failure closely mimicking what is seen clinically in dogs. Unfortunately, the subject mortality which occurred during our final pacing treatment (220bpm) prevents us from stating positively that VO$_2$ kinetics (TIP) may be used as a predictor of heart failure. Future studies of the same design will hopefully establish VO$_2$ kinetics as a valid quantifier for the severity of heart failure.
References


CHAPTER 4

CHANGES IN CANINE NEUROHORMONE LEVELS WITH INCREMENTAL INCREASES IN RAPID VENTRICULAR PACING

Introduction

There are four major neurohormonal systems involved in the pathophysiology of heart failure. These include the catecholamines from the sympathetic nervous system, renin-angiotensin-aldosterone triad, vasopressin, and atrial natriuretic peptide. (Travill 1992) Different researchers have separately documented the role of these neurohumoral responses to rapid ventricular pacing. (Travill et al., 1992; Perrella et al., 1992; Shen et al., 1996; Moe et al., 1990; Seymour et al., 1994; & Riegger et al., 1982) A commonality among different studies, which have been done to date, is the use of a rapid pacing model in dogs to induce the condition of heart failure. These studies have employed varying pacing rates, ranging from 180bpm to 260bpm, and varying pacing durations ranging from 10 to 21 days. (Travill et al., 1992; Perrella et al., 1992; & Redfield et al., 1993)

Redfield et al (1993) and Stevens et al (1995) initiated the use of 180 beats per minute for 10 days for the purpose of investigating hormonal response. This rate and duration mimics the early stages of heart failure and has provided insight into the
hormonal responses to cardiac insufficiency at this level. Severe heart failure has been imposed using higher pacing rates (220-260bpm) for a longer duration (3 weeks). Moe et al (1990) and Riegger et al (1982) have characterized various hormone responses to this severe state of failure.

Although the attempt to characterize hormonal response in early heart failure (180bpm) and severe heart failure (220-260bpm) is functionally significant, it is incomplete. Moderate pacing rates also need to be employed and investigated for their effect on hormone levels. Along with the effects studied at low and high pacing rates, the characterization of a moderate rate would more closely mimic the progression of heart failure as observed clinically. The purpose of this study was to investigate neurohormonal changes at three different pacing rates, 180, 200, and 220bpm, employed progressively, each for a 3-week interval. It is believed that this model more closely replicates the natural progression of disease observed in a clinical setting.

Materials and Methods

Subjects

Fourteen large (25-35 kg) mature, male, hound-type dogs were used in this study. Each dog was determined to be in good health by; appetite, general appearance and physical examination. The physical examination included auscultation of the heart and lungs, thoracic radiography, electrocardiography and echocardiography. The other primary inclusion criterion for this study was a willingness by the dog to run on a motorized treadmill while wearing a facemask.
Pacing Protocol

A magnetically programmable pacemaker was implanted, aseptically, to pace the right ventricle at various rates. (Redfield et al., 1993) Under anesthesia a small incision was made in the cervical region, and the pacing leads were threaded through the maxillary branch of the jugular vein, and impacted on the endocardium of the right ventricle. The trabeculae carneae were used to hold the leads in place. The pacemaker was interred subcutaneously in a neck pouch, and the incision was sutured closed.

The pacing protocol consisted of progressive increases in heart rate at three-week intervals. Pacing began at 180bpm for the first three weeks, and was increased to 200bpm for weeks 4 through 6, and 220bpm for weeks 7 through 9.

Shortening Fraction (ejection fraction):

Ejection fraction (EF) is the time-honored method of assessing ventricular function. It is the ratio of stroke volume (SV) to end-diastolic volume (EDV) of the left ventricle. EF is calculated by subtracting end-systolic volume (ESV) from EDV to obtain SV, and then dividing SV by EDV. In this study, left ventricular shortening fraction (SF) was used as a surrogate for EF. SF was calculated by 2-D directed M-mode echocardiography, in which EDD (end-diastolic diameter) and ESD (end-systolic diameter) were averaged over 3 cardiac cycles. The cursor was placed just apically to the mitral apparatus. EDD was measured at the beginning of the QRS complex, and ESD was measured at the peak of the T wave on the ECG. Both FS and EF have been shown to correlate closely with one another and with the severity of compromise of left ventricular function in heart failure. (Poole, 1997; Fortun et al, 1971) Twelve hours prior to each exercise session, the dog's pacemaker was inactivated and each dog received 0.8 mg/kg
(body weight) of Butorphanol subcutaneously. Fifteen minutes later, echocardiography was performed to determine SF. At the end of the procedure the pacemaker was re-activated.

**Collection of plasma and serum**

Resting levels of the endocrines; atrial natriuretic peptide (ANP), plasma renin activity (PRA), aldosterone, and anti-diuretic hormone (ADH) were assessed at baseline and at the end of each 3-week period of pacing. One hour prior to the collection of blood, each the dog was mildly sedated with Butorphanol (.08mg/kg body weight). Twenty-eight milliliters of whole blood was drawn from a jugular stick, and distributed into 4, 7 ml ethylene-diamine tetra-acetic (EDTA) tubes which were then placed immediately on ice. The tubes were centrifuged at 6,000 rpm’s at 4° C for ten minutes. Plasma from each tube was pipetted immediately into clean cryovials for storage at -80° C. An additional four milliliters of whole blood was drawn and placed into a serum tube remaining at room temperature for 30 minutes. The tube was then centrifuged at 6,000 rpm’s at 20° C for 10 minutes. The serum was pipetted into clean cryovials for storage at minus 80° C.

**Hormonal Assays**

Blood samples were assessed for ANP, PRA, ADH and aldosterone using radioimmunoassay techniques. Each of these hormones was measured using techniques previously described by Burnett et al (1986), Haber et al (1969), Sando et al (1978) and Morton et al (1975) respectively. Blood analysis was performed by Labcorp, Laboratory Corportation of America (1447 York Court, Burlington, NC 27215).
Catecholamine Assay

The analysis of plasma catecholamines was determined by High Pressure Liquid Chromatography (HPLC) method using electrochemical detection. The frozen plasma samples were assayed at the General Clinical Research Center (The Ohio State University Medical Center, Columbus Ohio), using methods described by Causon & Carruthers (1982) and analyzing equipment by Waters, (Waters Chromatography Division, 34 Maple St. Milford, MA 01757)

Data Analysis

The statistical analysis performed on the data was an analysis of variance with repeated measures. Mean values at baseline, 180bpm and 200bpm were compared using a sample of 14 subjects. Due to subject mortality the data presented for 220bpm was made up of a sample of six. The mean values for this treatment were plotted to help establish a trend line, but they were not included in the repeated measures. Bonferroni post-hoc tests were administered whenever the F-test indicated a p<0.05 for main effects.

Results

Not all of the fourteen dogs, which began this study, were able to complete the entire pacing protocol. Between the 6th and the 9th week period, when pacing had increased from 200bpm to 220bpm, eight dogs were removed from pacing and dropped from the study due to the severity of their symptoms. This left us with an n of 6 for the final treatment (220bpm).

Prior to the start of the study we had planned to treat the data with repeated measures analysis of variance (ANOVA). However, with the large loss in the number of subjects, it was decided to exclude the last treatment (220bpm) from the repeated
measures analysis and report the findings from this group in a descriptive manner to help illustrate any possible trend lines in the data. Any significance determined with the ANOVA was treated further with a Bonferroni multiple comparison post-hoc analysis.

Body weight in the dogs dropped from $30.5 \pm 1.03$ kg to $28.3 \pm 1.46$ kg from baseline to the end of 220bpm pacing period (9 weeks) respectively. This loss in body weight was not significant.

Shortening fraction was used as the quantifier for the severity of heart failure experienced with each pacing rate. Mean shortening fraction (SF) decreased with each increase in pacing rate. At 180bpm there was a significant decrease from the baseline of $35.5 \pm 1.36$ % to $25.0 \pm 1.42$ % ($p < .05$). The SF of $19.5 \pm 1.87$ % measured at 200bpm was significantly different from 180bpm and baseline ($p < .05$). At 220bpm the mean SF of $12.2 \pm 2.27$ % continued the downward trend from 180bpm and 200bpm (Fig. 4.1).
Figure 4.1. Changes in Shortening Fraction following increases in ventricular pacing rates

There were no significant (p < .05) changes in any of the neurohormones measured with the exception of Atrial Natriuretic Peptide (ANP). Plasma levels of ANP increased from 36.8 ± 2.55 (pg/ml) at baseline to 44.1 ± 2.98 (pg/ml) at the end of 3 weeks of pacing at 180bpm. Following 3 weeks at 200bpm, the level of ANP remained significantly (p < .05) elevated at 54.8 ± 5.53 (pg/ml). This increase appeared to remain level at 52.0 ± 11.58 (pg/ml) even though pacing was increased to 220bpm for 3 weeks.
The results for ANP, ADH, PRA and aldosterone are presented in Table 4.1 and figures 4.2 through 4.5 respectively.

Table 4.1. | Characteristics of Neurohormones and Catecholamines During Development of Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=14)</th>
<th>180bpm (n=14)</th>
<th>200bpm (n=14)</th>
<th>220bpm (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atrial Natriuretic Peptide (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.8 ± 2.55</td>
<td>44.1 ± 2.98*</td>
<td>54.8 ± 5.53*</td>
<td>52.0 ± 11.58</td>
</tr>
<tr>
<td><strong>Antidiuretic Hormone (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0 ± 0.58</td>
<td>5.0 ± 1.02</td>
<td>2.4 ± 0.62</td>
<td>4.2 ± 0.82</td>
</tr>
<tr>
<td><strong>Plasma Renin Activity (ng/ml/hr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0 ± 0.25</td>
<td>2.4 ± 0.36</td>
<td>3.2 ± 0.66</td>
<td>6.1 ± 2.87</td>
</tr>
<tr>
<td><strong>Aldosterone (ng/dl)</strong></td>
<td>3.4 ± 1.14</td>
<td>7.0 ± 2.06</td>
<td>10.3 ± 3.50</td>
<td>54.4 ± 42.62</td>
</tr>
<tr>
<td><strong>Norepinephrine (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>451.1 ± 46.15</td>
<td>678.4 ± 69.83*</td>
<td>855.9 ± 99.64*</td>
<td>1002.8 ± 267.60</td>
</tr>
<tr>
<td><strong>Epinephrine (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>668.9 ± 179.46</td>
<td>619.4 ± 116.25</td>
<td>959.4 ± 256.57</td>
<td>258.8 ± 67.88</td>
</tr>
<tr>
<td><strong>Dopamine (pg/ml)</strong></td>
<td>44.2 ± 7.26</td>
<td>67.1 ± 8.71</td>
<td>70.4 ± 10.38*</td>
<td>117.5 ± 43.80</td>
</tr>
</tbody>
</table>

Values = mean ± SE
*Significantly different from baseline (p < .05)
Values = mean ± SE
*Significantly different from baseline (p < .05)

**Figure 4.2.** ANP values following increases in ventricular pacing rates
Figure 4.3. ADH levels following increases in ventricular pacing rates

Values = mean ± SE

Figure 4.4. PRA levels following increases in ventricular pacing rates

Values = mean ± SE
Changes in catecholamines with the increases in pacing rate included a steady incline in norepinephrine levels (pg/ml). The increase from a baseline value of 451.1 ± 46.15 to 678.4 ± 69.83 at 180bpm was significant (p < .05). This change in norepinephrine remained significant, and increased further to 855.9 ± 99.64 at the pacing rate of 200bpm. This upward trend continued at 220bpm reaching a value of 1002.8 ± 267.6 (Table 4.1, Fig. 4.6).

Epinephrine (pg/ml) levels were changed with each change in pacing rate, however without any significance or identifiable trend (Table 4.1, Fig. 4.7). Dopamine (pg/ml) also changed with each increase in pacing rate, showing an upward trend from baseline to 220bpm. The measured values were: 44.2 ± 7.26 (baseline), 67.1 ± 8.71
(180bpm), 70.4 ± 10.38 (200bpm), and 117 ± 43.80 (220bpm). The change in dopamine from baseline to 200bpm was significant (p < .05) (Table 4.1, Fig. 4.8).

Values = mean ± SE
*Significantly different from baseline (p < .05)

Figure 4.6. Norepinephrine Levels Following Increases in Ventricular Pacing Rates
Values = mean ± SE

**Figure 4.7.** Epinephrine Levels Following Increases in Ventricular Pacing Rates
Figure 4.8.  Dopamine Levels Following Increases in Ventricular Pacing Rates

Discussion:

Previous researchers have used various pacing rates and durations to elicit heart failure in dogs. (Parrella et al., 1992; Moe et al., 1990; Redfield et al., 1993; Stevens et al., 1995; & Riegger et al., 1982) With the exception of Redfield (1993) and Stevens (1995), who employed 180bpm to investigate the early effects of heart failure, previous studies have used rates of 200bpm or greater with the intent of producing severe heart failure. The purpose of this study was to progress the dogs, in a step-wise fashion, through the evolution of heart failure (mild, moderate and severe) in order to investigate corresponding changes in neuroendocrines.

Shortening fraction (SF) was used as the criteria to mark the evolution of heart failure. (Burkett et al., 1994) The results indicate that we were successful in eliciting a
graded approach to severe heart failure. Normal, resting SF for a dog is 35 % and our baseline data corresponded to this. Three weeks of pacing at 180bpm reduced SF to 25 % (a 40% reduction). Increasing the pacing rate to 200bpm for the next 3-weeks reduced SF even further to 19.5 %. The trend of decreasing SF with an increase in pacing rate continued when the rate went to 220bpm. After 3-weeks at this rate SF fell to 12 % indicating severe or overt heart failure. Our model clearly demonstrated an incremental increase in the severity of heart failure, from mild to moderate to severe with 3-week incremental increases in ventricular pacing rate.

As reported in the data analysis, we suffered from subject mortality as a result of the dogs reaching what we established as end point criteria. Our criteria for removing a dog from the study consisted of a SF of <17% and/or physical signs (i.e., ascites, crackles, shadows on the radiograph, and lethargy).

Atrial Natriuretic Peptide (ANP) is a rapidly metabolized hormone. Precautions were taken to prevent its rapid breakdown in the drawn blood sample, by chilling the blood collecting tubes on ice and immediately storing the cryovials at -80 degrees C. Additionally, aprotinin (protease inhibitor) was added to the blood collection tube for prevention of further breakdown. (Riegger et al 1988)

Our ANP results displayed an increase in value from baseline through 180bpm and 200bpm. At the rate of 220bpm, the values seemingly plateau. The response of ANP, in this study, was similar to the response elicited by other researchers. (Riegger et al., 1988; & Moe et al., 1990) However, the gradual increase in pacing rate employed in this study, resulted in an elongation of the rate of rise in the levels of ANP compared to prior research.
An increase in antidiuretic hormone (ADH) is known to result in salt and water re-absorption in the kidney. In clinical cases of heart failure, ADH is often elevated, and we expected the same response in this study. Our results showed a quick rise in ADH at 180bpm followed by a drop at 200bpm. Levels rose again at 220bpm. This pattern of change, as well as, the large variability (SE) in ADH between dogs at each pacing rate prevented finding any significant difference between treatments. However, our findings are consistent with what was found in a study by Travill (1992).

Plasma renin activity (PRA) steadily rose with each increase in ventricular pacing. Our end results were similar to those achieved by Riegger et al in 1984. This previous study demonstrated that PRA increases over time, peaking at day 7 of pacing at 240bpm. Our model induced heart failure in a graded manner over a 63-day period. Therefore, our increase in PRA was not as rapid as previous studies have shown. However, our end stage or severe heart failure (220bpm) data correspond to what was found in severe heart failure by others. (Riegger, 1984; & Travill, 1992)

The observed catecholamine response to increasing pacing rates over time was as expected, except for epinephrine. Norepinephrine and dopamine followed a pattern similar to that reported by Spinale (1996). As the severity of heart failure increased (pacing rate) there was a corresponding rise in these two catecholamines.

Our epinephrine results were non-conforming to those found in previous literature. Our values at baseline, 180bpm and 200bpm were all well above previous reports (Spinale, 1996 & Riegger, 1988). We believe our results were affected by the excited behavior demonstrated by the dogs when removed from their kennel for blood sampling. An insufficient amount of time was allowed for them to “settle down” prior to
drawing blood. Our relatively low value for epinephrine at the final pacing rate (220bpm) may be attributed to the lethargy associated with severe heart failure.

The results of this study are consistent with the hormonal activation known to occur with rapid ventricular pacing induced heart failure. ANP immediately increases in response to changes in volume and pressure detected in the atria and carotid sinus. This increase in plasma ANP delays the onset of the vasoconstrictive mechanisms (renin-angiotensin-aldosterone triad) as seen by Redfield (1993). The progressive increase in pacing rate, over time, demonstrated a more gradual rise in the sympathetic nervous system (norepinephrine) and vasoconstrictive mechanisms compared to studies employing faster rates for shorter durations. In clinical cases, the development of heart failure requires time and chronic bombardment of these hormones to produce its associated signs and symptoms. Our protocol, employing a gradual increase in pacing rate over a relatively prolonged period promises to be a sound model for replicating the natural course of developing heart failure.

Reference:


SUMMARY

The rapid ventricular pacing model has been extensively research and is known to produce heart failure in dogs. This study increased the pacing rate over time in an attempt to elicit an evolution of disease ending in severe heart failure. Similar to that seen in the clinical setting. Shortening fraction was used as the quantifying index. We concluded that our model successfully mimicked the evolution of heart failure progressing from mild to moderate to severe.

The findings from the exercise portion of the study were important because there has been no documentation of oxygen consumption and rapid ventricular pacing in the literature to date. Unfortunately, subject mortality limited our drawn conclusions. However, future studies involving oxygen consumption and the use of this pacing model will evolve from these initial findings and establish the validity and reliability of this protocol.

The hormone portion of the study was designed to document the changes that occur with step-wise increases in rapid ventricular pacing. We accomplished this goal in part. Again, subject mortality in addition to the variability in the hormones measured, limited our significant results and therefore our conclusions.
BIBLIOGRAPHY


