Plasma N-terminal proatrial natriuretic peptide concentration in cats with hypertrophic cardiomyopathy

by

Heidi Norma MacLean, DVM

Virginia Polytechnic Institute and State University

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Clinical Sciences
March 12, 2004
Blacksburg, VA

Jonathan A. Abbott, DVM, Chair
William R. Huckle, MS, PhD
R. Lee Pyle, VMD, MS

Keywords: hypertrophic cardiomyopathy, atrial natriuretic peptide, diastolic dysfunction, feline

Copyright 2004, Heidi N. MacLean
Objective: We sought to determine N-terminal proatrial natriuretic peptide concentrations [Nt-proANP] in plasma from cats with hypertrophic cardiomyopathy (HCM). Secondarily, we wished to evaluate the relationship between [Nt-proANP] and echocardiographic variables.

Methods: Venous blood samples were obtained from seventeen cats with HCM and from nineteen healthy cats. Plasma [Nt-proANP] was determined using an ELISA assay. The relationship between plasma [Nt-proANP] and M-mode, 2-dimensional and Doppler echocardiographic variables was evaluated. Cats that were hyperthyroid or had evidence of renal disease were excluded from the study.

Results: The mean plasma [Nt-proANP] was higher in cats with HCM (3.81 +/- 1.23 pmol/l) than in control cats (3.08 +/- 1.41 pmol/l); however, this difference was not statistically significant (p=0.17). There was a significant correlation between plasma [Nt-proANP] and left ventricular posterior wall thickness (r = 0.42; p=0.01). Additionally, plasma [Nt-proANP] was correlated with left atrial size (r = 0.35; p=0.03). A linear regression model was developed to further explore these relationships. LAs2D
and LVPWd had an interactive effect on plasma [Nt-proANP] \( (R^2 = 0.2737; \ p = 0.02) \). There was no correlation between any other echocardiographic variable and plasma [Nt-proANP]. There was no correlation between plasma [Nt-proANP] and heart rate (HR), body-weight, or age.

**Conclusions:** Cats with HCM do not have significantly higher plasma [Nt-proANP] than normal cats. There was a significant linear relationship between [Nt-proANP] and LAs2D, LVPWd and the model that described their interaction.
ACKNOWLEDGEMENTS

The author would like to thank the Virginia Veterinary Medical Association for providing funding for this project. The author also wishes to recognize the VTH hospital technicians for their assistance and patience. As well, the author would like to thank Mr. Daniel Ward for help with the statistical analysis, Dr. R. Lee Pyle and Dr. William R. Huckle, members of the committee, for their suggestions and guidance throughout the study and Dr. David D. Sisson for his assistance with peptide analysis.

In addition, the author wishes to thank her family for all their support and guidance throughout the years; without them, none of this would have been possible. Finally, the author extends a special thank you to Dr. Jonathan A. Abbott for his mentorship, friendship, guidance and patience, not only during the project, but over the past three years.
TABLE OF CONTENTS

Abstract ................................................................. ii
Acknowledgements .................................................... iv
Table of Contents ...................................................... v
List of Figures .......................................................... vi
List of Abbreviations .................................................. vii
Introduction ............................................................. 1
Chapter I: Literature Review .......................................... 4
I. Atrial Natriuretic Peptide ........................................... 4
   A. Introduction ..................................................... 4
   B. ANP Synthesis and release ................................. 5
   C. Natriuretic peptide receptors ............................... 6
   D. Physiologic actions of ANP ................................. 8
II. Diastolic function, dysfunction and HCM ........................ 9
   A. Diastolic function ............................................. 10
   B. Diastolic dysfunction and HCM ............................ 16
III: Neurohormonal activity & cardiac disease .................... 20
Chapter II: Plasma N-terminal proatrial natriuretic peptide
            concentration in cats with hypertrophic cardiomyopathy.. 25
   A. Abstract .................................................. 25
   B. Introduction .............................................. 27
   C. Materials and methods .................................... 28
   D. Results .................................................. 33
   E. Discussion ................................................ 34
Chapter III: Conclusions ............................................ 40
Footnotes ............................................................... 41
References .............................................................. 42
Appendix I: Figures .................................................. 47
  Figure 1 ...................................................... 47
  Figure 2 ...................................................... 48
  Figure 3 ...................................................... 49
  Figure 4 ...................................................... 50
Vita ................................................................. 51
LIST OF FIGURES

Figure 1.  Page 47.
Box and whisker plot of plasma [Nt-proANP] in 17 cats with hypertrophic cardiomyopathy (HCM) and 19 control cats.

Figure 2.  Page 48.
A scatterplot that demonstrates the correlation between plasma Nt-proANP concentration [Nt-proANP] and left ventricular posterior wall diastolic thickness (LVPWd) as measured from a long-axis right parasternal transducer position in 17 cats with HCM and 19 control cats.

Figure 3.  Page 49.
A scatterplot that demonstrates the correlation between plasma Nt-proANP concentration [Nt-proANP] and left atrial systolic dimension (LAs2D) as measured from a long-axis right parasternal transducer position in 17 cats with HCM and 19 control cats.

Figure 4.  Page 50.
Regression model of the interactive relationship of LAs2D (measured in centimeters) and LVPWd (measured in centimeters) with plasma [Nt-proANP] in 17 cats with HCM and 19 control cats.
LIST OF ABBREVIATIONS

AA    amino acid
ADH    antidiuretic hormone
ANP    atrial natriuretic peptide
[ANP]  atrial natriuretic peptide concentration
BNP    brain natriuretic peptide
CGMP   cyclic guanosine monophosphate
CHF    congestive heart failure
CNP    c-type natriuretic peptide
CNS    central nervous system
cTn-I   cardiac troponin I
cTn-T   cardiac troponin T
DCM    dilated cardiomyopathy
EIA    enzyme immunoassay
ET-1    endothelin-1
HCM    hypertrophic cardiomyopathy
HOOCM   hypertrophic obstructive cardiomyopathy
HR     heart rate
IVRT    isovolumic relaxation time
IVSd    interventricular septal thickness during diastole
         measured by M-mode echocardiography
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSs</td>
<td>Interventricular septal thickness during systole measured by M-mode echocardiography</td>
</tr>
<tr>
<td>LADs</td>
<td>Left atrial systolic dimension measured by M-mode echocardiography</td>
</tr>
<tr>
<td>LAmin</td>
<td>Minimal left atrial dimension measured by M-mode echocardiography</td>
</tr>
<tr>
<td>LAmax</td>
<td>Maximal left atrial dimension measured by M-mode echocardiography</td>
</tr>
<tr>
<td>LAs2D</td>
<td>Left atrial systolic dimension from a right parasternal 2-dimensional echocardiographic image</td>
</tr>
<tr>
<td>LVPWd</td>
<td>Left ventricular posterior wall thickness during diastole measured by M-mode echocardiography</td>
</tr>
<tr>
<td>LVPWs</td>
<td>Left ventricular posterior wall thickness during systole measured by M-mode echocardiography</td>
</tr>
<tr>
<td>MVA</td>
<td>Peak velocity of late diastolic ventricular filling measured by pulsed-wave Doppler echocardiography</td>
</tr>
<tr>
<td>MVE</td>
<td>Peak velocity of early diastolic ventricular filling measured by pulsed-wave Doppler echocardiography</td>
</tr>
<tr>
<td>MVE/DT</td>
<td>Rate of deceleration of mitral valve inflow</td>
</tr>
<tr>
<td>NPR-A</td>
<td>A-type natriuretic peptide receptor</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NPR-B</td>
<td>B-type natriuretic peptide receptor</td>
</tr>
<tr>
<td>NPR-C</td>
<td>C-type natriuretic peptide receptor</td>
</tr>
<tr>
<td>Nt-proANP</td>
<td>N-terminal proatrial natriuretic peptide</td>
</tr>
<tr>
<td>[Nt-proANP]</td>
<td>N-terminal proatrial natriuretic peptide concentration</td>
</tr>
<tr>
<td>PCWP</td>
<td>Pulmonary capillary wedge pressure</td>
</tr>
<tr>
<td>preproANP</td>
<td>Pre-proatrial natriuretic peptide</td>
</tr>
<tr>
<td>proANP</td>
<td>Proatrial natriuretic peptide</td>
</tr>
<tr>
<td>PVD</td>
<td>Pulmonary vein Doppler</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin-angiotension-aldosterone-system</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>SAM</td>
<td>Systolic anterior motion of the mitral valve</td>
</tr>
<tr>
<td>SERCA</td>
<td>Sarcoendoplasmic reticulum calcium ATPase</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>TDI</td>
<td>Tissue Doppler imaging</td>
</tr>
</tbody>
</table>
INTRODUCTION

HCM is the most prevalent cardiac disease in adult cats. It is characterized by hypertrophy of a nondilated ventricle in the absence of identifiable cause. In people, HCM is a genetic disease and is inherited as an autosomal dominant trait. HCM is heritable in Maine Coon Cats and familial forms of the disease are recognized in other breeds. HCM is a major cause of morbidity and mortality in cats. It is most often manifest clinically through signs of congestive heart failure, systemic thromboembolism, and sudden death. HCM is often detected in a subclinical form when abnormalities detected on routine physical examination prompt echocardiographic examination. Feline HCM is characterized by left ventricular diastolic dysfunction and has a heterogeneous morphologic expression. Most often there is symmetrical hypertrophy that involves the interventricular septum and left ventricular free wall. Less commonly, hypertrophy is asymmetrical or is segmental and involves only a portion of the interventricular septum or free wall. Feline HCM is similar to the disorder seen in humans with regards to some clinical and pathological features. However, in humans with HCM, myocardial hypertrophy is typically asymmetrical with interventricular septal thickening that is disproportionate to that of the left ventricular posterior wall. In addition, HCM in humans is characterized by specific myocardial cellular disorganization. Small foci of cell-to-cell disarray is seen in cats with HCM, however, the mean area of disorganization in both the interventricular septum and left ventricular free wall is greater in human patients with HCM than in feline patients.
HCM results in diastolic dysfunction that follows as a consequence of impaired myocardial relaxation and reduced ventricular compliance. This can lead to elevated filling pressures, left atrial enlargement and potentially, the development of left-sided congestive heart failure. In people, left atrial enlargement is a risk factor for the development of atrial thrombi; stretching of the left atrium can alter the endothelium and make it more susceptible to platelet adherence and thrombus formation.[4, 16] The prognosis associated with feline HCM is highly variable and the factors that determine clinical outcome are incompletely understood. It is known, however, that development of congestive heart failure and occurrence of systemic thromboembolism have a negative impact on prognosis. [12] Echocardiographic evidence of left atrial enlargement is generally believed to reflect severe diastolic dysfunction and to represent a harbinger of morbid events.

Recently, there has been a growing interest in the measurement of natriuretic peptides in human patients with HCM and in those with congestive heart failure. During the past few years, plasma concentrations of natriuretic peptides have gained widespread acceptance as a diagnostic and prognostic marker for human patients with congestive heart failure.[17-19] As well, in multiple studies, plasma atrial natriuretic peptide concentration [ANP] has been found to be markedly elevated in human patients with heart diseases such as aortic or mitral stenosis, [20-22] dilated cardiomyopathy (DCM)[20, 22] or heart disease as a result of tachycardia.[23, 24] Recently, several studies have sought to assess the importance of ANP in the diagnosis and/or prognosis of human patients with HCM.[25-30]
ANP is a polypeptide that has natriuretic, diuretic and vasodilatory properties and contributes to the regulation of blood pressure and fluid homeostasis. ANP is produced and secreted predominantly by the atria in response to volume or pressure overload. Plasma [ANP] is increased in patients with congestive heart failure and [ANP] correlates well with left ventricular end-diastolic pressures and atrial pressures.[31-34] Therefore, the relationship between plasma [ANP] and the severity of heart failure likely reflects, to some extent, the higher atrial pressure associated with more severe heart failure.[30]

Recently, the prognostic role of plasma [ANP] has been investigated in human patients with HCM. Patients with high plasma [ANP] had a poorer prognosis than those patients with low plasma [ANP]. As well, plasma [ANP] was the most important risk factor for all adverse cardiovascular events including sudden cardiac death, heart failure, peripheral embolism and ischemic stroke.[35]

Unfortunately, there is a paucity of information regarding neuroendocrine activation in cats with heart disease. Furthermore, the clinical outcome in feline patients with HCM is difficult to predict. The proposed investigation will test the hypothesis that plasma [Nt-proANP] in feline patients with HCM exceeds that in healthy cats. The primary study objective was to determine plasma [Nt-proANP] in cats with HCM and in a control sample of healthy cats. Secondarily, we wished to evaluate the relationship between [Nt-proANP] and echocardiographic variables. This study will help elucidate the clinical importance of plasma [Nt-proANP] in feline HCM and will form the basis for studies that will define the prognostic relevance of plasma [Nt-proANP] in patients with this disorder.
CHAPTER I

Literature Review

I. Atrial Natriuretic Peptide

A. Introduction

In 1956, electron dense granules were identified in the atrial myocytes of guinea pigs. [36] Similar granules were later found in atrial myocytes of other species and this led to the landmark study by de Bold in 1981 which demonstrated a natriuretic and diuretic effect of rat atrial tissue;[37] a 28 amino acid (AA) peptide was isolated from those extracts and the structure of ANP was identified in 1984.[38] This discovery has led to the description of a family of structurally similar but genetically distinct peptides known as the natriuretic peptide family.

The natriuretic peptide family consists of three peptides: ANP, brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These peptides are thought to prevent hypervolemia and hypertension through their natriuretic and diuretic actions. These peptides also act as vasodilators and have antimitogenic effects on cardiovascular tissues. [39] Each peptide originates from a precursor prohormone that is encoded by a separate gene. The regulation of secretion and the tissue distribution of each is unique.[40]
B. ANP synthesis and release

In normal cats, ANP gene expression and ANP production are mostly restricted to the atrial and auricular cardiomyocytes, particularly those located in close proximity to the endocardial surface. Only small amounts of ANP are found in ventricular myocytes.[41]

In humans, ANP is a 28 amino acid (AA) peptide that is produced and stored principally within atrial cytoplasmic granules as a prohormone.[42-44] ANP is rapidly released from atrial myocytes in response to elevations in atrial pressure or stretch.[39, 40] It is secreted, mostly from a store of previously formed hormone within the atrial myocyte, while a much smaller amount is derived from newly synthesized hormone. The regulation of ANP, therefore, occurs mainly at the level of hormone secretion and a large decrease in stored ANP must occur before transcriptional activity begins and new ANP is created.[39, 40]

In humans, ANP is synthesized as preproANP which contains 151 AA’s. A 25-AA signal peptide is cleaved during cellular transport of preproANP and the precursor peptide, proANP, which consists of 126 AA’s is then stored in atrial myocytes. Cleavage of this peptide by atriopeptidase results in a 98 AA N-terminal fragment (Nt-proANP) and a 28 AA C-terminal fragment - mature ANP.[39, 40] Both fragments circulate in plasma and there is some evidence to suggest that the N-terminal fragment has biological actions similar to ANP. [45] The nucleotide and amino acid sequence of ANP has been revealed for many species,
including horses, sheep, rabbits, rats, dogs, cattle, pigs, and cats.[46] The sequence for feline preproANP consists of 153 AA’s. The signal peptide consists of 25 AA’s, the Nt-proANP consists of 98 amino acids and the C-terminal proANP consists of 30 AA’s. The feline Nt-proANP (98 AA’s) is highly conserved; it is 94% homologous with the sequence in horses and humans. The plasma half-life of Nt-proANP is approximately 8 times longer than that of C-terminal ANP (2-5 minutes) and therefore, its plasma concentration is about 10 times higher than that of C-terminal ANP. Feline ANP is similar to human ANP, differing only by two arginine residues.[46]

Both atrial stretch and elevated atrial pressures have been shown to be primary stimuli for ANP release.[47-49] Several hormones and neurotransmitters also serve as stimuli for secretion of ANP; these include vasoactive substances such as angiotensin II, catecholamines, endothelin and vasopressin. Fibroblast growth factor, hypoxia, substance P and transforming growth factor –B also stimulate ANP release.[48]

ANP secretion is mediated by intracellular calcium concentrations; calcium influx via T-type channels increase the release of ANP from atrial myocytes while calcium influx via L-Type calcium channels inhibits ANP release.[50] ANP secretion is also inhibited by nitric oxide.[51]

C. Natriuretic Peptide Receptors

ANP is secreted into the bloodstream and is a ligand for genetically distinct A-type natriuretic peptide receptors (NPR-A)[48, 49] located in
endothelium, smooth muscle, cardiomyocytes, fibroblasts, coronary vessels, lung, kidney,[48] eye, adrenal glands, adipose tissue, pregnant uterus and placenta.[49]

There are three different natriuretic peptide receptors (NPR) -A, B and C. NPR-A and B have about 44% homology in the ligand-binding domain.[52] NPR-A binds both ANP and BNP with preference for ANP; NPR-B binds CNP.[40, 48, 49] NPR -A is the most abundant NPR in the large blood vessels while the NPR-B predominates in the brain; both receptors are present in the kidney.[40] NPR-A and NPR-B activation leads to guanyl cyclase activation and formation of the second messenger cyclic guanosine monophosphate (cGMP).[49] cGMP exerts a biological effect by activating protein kinase G and other kinases that phosphorylate various regulatory proteins and enzymes.[48, 53]

NPR-C is a receptor involved in the clearance of circulating ANP, BNP and CNP. ANP binds to this receptor, is internalized and enzymatically degraded by lysosomal hydrolysis; NPR-C then returns to the cell surface. Approximately equal amounts of circulating ANP are inactivated by neutral endopeptidases located in the renal tubular cells and vascular cells.[26, 49, 54, 55] Together these systems clear approximately 75% of the ANP in the circulation.[40, 48]

Clearance mainly occurs in the lungs, liver and kidneys but also occurs in the heart, vascular endothelium and smooth muscle. The plasma half-life of ANP is approximately 1-2 minutes.[48] The inactive Nt- proANP has no specific clearance receptors and therefore, it has a longer half-life, and circulates in the plasma in much higher concentrations than ANP.[49]
D. Physiologic actions of ANP

The principal function of ANP (and BNP) is to protect the cardiovascular system from volume overload. Volume overload increases plasma concentrations of ANP.[48] ANP causes a reduction in peripheral vascular resistance and lowers blood pressure. This decrease in blood pressure results in part from a decrease in cardiac preload caused by shifting of fluid from the intravascular to extravascular compartment due to increased vascular endothelial permeability. As well, ANP increases venous capacitance and promotes natriuresis and diuresis due to its direct effect on the kidney and suppression of the renin angiotensin aldosterone system (RAAS).[40]

ANP decreases sympathetic outflow from the central nervous system and inhibits the release of norepinephrine from the peripheral sympathetic nerve endings and desensitizes the baroreceptors. ANP reduces the activation threshold of the vagal afferents, and effectively prevents the reflex tachycardia and vasoconstriction that occurs when blood volume decreases and mean arterial pressure declines.[39, 40]

ANP stimulates dilation of afferent renal arterioles and constriction of efferent arterioles; this leads to increased pressure within the glomerular capillaries and an increased glomerular filtration rate. This effect does not last as long as the natriuretic action of ANP and thus another mechanism is involved. ANP directly inhibits sodium transport in the proximal tubule and in the inner
medullary collecting duct and therefore promotes natriuresis.[40, 49, 56] As well, the natriuretic effect of ANP in the absence of an elevated glomerular filtration rate may be the result of locally produced ANP (i.e. urodilatin) acting through a paracrine mechanism or systemic ANP. ANP inhibits angiotensin II stimulated sodium and water transport in the proximal convoluted tubules and inhibits aldosterone release from the adrenal glands and renal renin release as well.[49, 56] In the cortical collecting ducts, ANP inhibits tubular water transport by antagonizing antidiuretic hormone (ADH).[51] In the inner medullary collecting duct, it stimulates cGMP production but prevents sodium absorption. The volume contracting and vasodilating ability of ANP reduces systemic vascular resistance and decreases intracardiac filling pressures. Natriuretic peptides have antimitogenic and antiproliferative actions in the cardiovascular system; they inhibit cell growth, proliferation, and cardiomyocyte hypertrophy.[39]

ANP is produced in the central nervous system (CNS)(particularly the hypothalamus).[40, 57] Natriuretic peptides act in the brainstem to decrease sympathetic vasoconstriction and reduce activation threshold of vagal efferents; they therefore mitigate the reflex tachycardia and vasoconstriction that normally accompanies decreases in blood volume.[48] ANP does not cross the blood-brain barrier but locally secreted ANP inhibits secretion of ADH and corticotropin through effects on the brain and pituitary gland. ANP receptors located adjacent to the third ventricle bind to ANP and mediate salt appetite and thirst. All of these effects suggest that there are coordinated actions of the peripheral and
central control mechanisms for plasma volume regulation and electrolyte homeostasis.[40]

II. Diastolic function, dysfunction and HCM

A. Diastolic function

The cardiac cycle consists of two phases: systole and diastole. In Greek, diastole means “to send apart” and, for clinical purposes, begins with semilunar valve closure and ends with closure of the atroventricular valves.[58, 59] According to Brutsaert, the traditional definition of diastole should be questioned; he suggested that systole should include both contraction and relaxation and that diastasis is the period of passive filling. However, the traditional, clinical definition is more clinically familiar.[60]

On a cellular level, diastole is defined as the process by which ATP hydrolysis results in unlinking of actin-myosin crossbridges and a return of the sarcomeres to their pre-contractile configuration.[61] Diastole is intricately associated with cellular calcium handling. At the end of systole, the calcium ions release from their binding sites on troponin C and therefore cross-bridge cycling cannot occur and diastole begins. Calcium is removed from the cytoplasm by three different mechanisms. The most important is the sarcoendoplasmic reticulum calcium ATPase (SERCA). For each molecule of ATP, two calcium ions are transported into the sarcoplasmic reticulum (SR).[58]

Phospholamban, a pentamer protein, is the major regulator of the SERCA. The activity of phospholamban is regulated by its state of phosphorylation. Beta adrenergic
stimulation leads to phosphorylation of phospholamban and this enhances the uptake of calcium by SERCA into the SR and increases the rate of myocardial relaxation. The second mechanism of calcium removal is a calcium ATPase located within the sarcolemmal membrane, and the third is the sodium/calcium exchanger located in the sarcolemmal membrane. This exchanger transports calcium out of the cytosol into the extracellular space in exchange for one sodium molecule.[58] This process occurs in some cells while others are still undergoing contraction. Therefore, cellular diastole may begin while left ventricular pressure is still rising.[61]

Diastole is a complex process that is determined by numerous interrelated factors that include rate of left ventricular myocardial relaxation, diastolic suction, pericardial restraint, ventricular interaction, viscoelastic forces and atrial transport. The two major determinants of left ventricular filling are ventricular relaxation and chamber compliance.[62] Myocardial relaxation is the process whereby the myocardium returns to its initial force and length conditions. The onset of myocardial relaxation is difficult to define because it is the terminal phase of systole.[63] Myocardial relaxation comprises the majority of left ventricular (LV) ejection, left ventricular pressure decrease and rapid ventricular filling.[64] The onset of left ventricular relaxation occurs early in systole, after about 16% of ejection is complete.[65] Rapid ventricular filling occurs after the mitral valve opens and is modulated by myocardial relaxation. As mentioned, myocardial relaxation is an energy dependent process that relies on ATP hydrolysis and the sequestration of calcium in the SR.[66] In normal individuals, relaxation will be completed at minimal left ventricular pressures during rapid ventricular filling (}
following the opening of the mitral valve).[59] The process of relaxation is influenced by the interaction of several factors including (1) loading conditions, (2) inactivation (the decay of active force generation) and (3) the uniformity of distribution of loading conditions.[60, 67]

Quantitative description of myocardial relaxation is difficult because neither its onset nor completion can be defined. Clinical indices of relaxation are typically derived from description of the isovolumic phase of relaxation. The most commonly used index is known as tau, the time constant of relaxation. Tau is derived by fitting a monoexponential curve to the diastolic isovolumic left ventricular pressure trace. As heart rate increases, tau normally shortens, and results in decreased left ventricular filling pressure. Limitations of tau include the assumption that the left ventricular pressure will approach 0 mmHg, the fact that the decrease in pressure does not always follow a monoexponential curve and finally, that tau does not take into account the time of onset of relaxation.[59] Left ventricular isovolumic relaxation time (IVRT) can be measured with echocardiography. It is measured from the time of aortic valve closure to the opening click artifact of the mitral valve as seen by spectral Doppler. It is a nonspecific measure that is influenced by the relaxation rate as well as by other factors that influence the closure of the aortic valve and the pressure within the left atrium. Increased left atrial pressure causes premature mitral valve opening and shortened IVRT, whereas volume depletion and decreased left atrial pressure have the opposite effect.[68] In a study by Schober et al., significant correlation was found between tau and IVRT in healthy anesthetized cats. Similar to findings in normal dogs and humans with and without heart
disease, their study showed that IVRT is a useful index of ventricular relaxation.[69] A study by Golden et al. demonstrated that relaxation half-time (a measure of isovolumic relaxation) is longer in cats with HCM than in normal cats.[70]

The second phase of diastole is the rapid filling phase. This extends from mitral valve opening to the time when the left ventricular filling reaches its peak and is influenced by myocardial relaxation. In normal individuals, about 75% of LV filling occurs during early diastole when the transmitral pressure gradient is at its highest level.[68] During IVRT, the previous depolarization of the myocardium causes active relaxation of the myocardium as calcium is resequestered into the SR, and to a rapid increase in left ventricular diastolic volume. Myocardial relaxation in the face of constant ventricular blood mass leads to an absolute decrease in left ventricular chamber pressure. This pressure drop causes the left ventricular pressure to fall below that of the left atrium, ending IVRT and resulting in mitral valve opening.[59] This pressure gradient as well as the elastic recoil and the suction effect of the left ventricle allow for early rapid ventricular filling.[59, 61] Elastic recoil begins at the end of myocardial contraction when the myofibers have been shortened by active tension to a length that is less than their equilibrium length, most likely as a result of their connective tissue matrix.[63] As well, the peak filling rate is influenced by the viscoelastic properties of the myocardium and the passive compliance of the myocardium.[59] As blood flows into the left ventricle, the ventricular cavity enlarges.

The rapid filling phase can be measured by many methods including: digitized M-mode echocardiography, radionuclide angiography, cineangiography, and Doppler
echocardiography. To date, the most commonly used method for assessing diastolic function is the use of pulsed-wave Doppler echocardiography to evaluate mitral and pulmonary vein inflow velocities. Echocardiography does not measure myocardial relaxation as such, but estimates the degree of alteration of left ventricular function. Numerous factors including atrial and ventricular relaxation, contraction, compliance and loading conditions affect Doppler indices of diastolic function.[71] Analogous measurements can be made on both sides of the heart. When the mitral and tricuspid valves open, blood flow accelerates to peak filling velocity; this produces a wave on the Doppler spectrogram known as the E wave. Inflow velocity decelerates at a rate that is dependent on the rate of increase in left ventricular pressure. It is determined by a number of factors including left atrial/ left ventricular pressure gradient at mitral valve opening, left atrial and left ventricular compliance, rate of left ventricular relaxation, pericardial restraint, ventricular interactions and viscoelastic forces of the myocardium.[62] At some point, myocardial relaxation ends and any further increase in ventricular chamber size depends on passive compliance or stiffness of the myocardium.[72] Diastolic stiffness increases as left ventricular volume increases; therefore, there is a curvilinear relationship between pressure and volume. Due to this curvilinear relationship, quantification of myocardial compliance is difficult.[59]

Toward the end of diastole, the left atrium contracts resulting in a left ventricular /left atrial pressure gradient that contributes to left ventricular filling; this is seen as the A wave on pulsed-wave mitral inflow Doppler. The A wave occurs following the E wave and after left ventricular relaxation is complete, and therefore depends on left
ventricular chamber compliance, atrial volume and atrial contractility.[62]
Unfortunately, this method is not ideal. The wave pattern seen in normal individuals
often cannot be differentiated from individuals with impaired left ventricular chamber
compliance and therefore, the findings must be interpreted in context of other diagnostic
results.[61]

Pulmonary vein Doppler (PVD) is used as an adjunct to mitral inflow Doppler.
The velocity flow reversal during atrial contraction can provide clinically useful
information.[73] The duration of blood flow across the mitral valve indexed to duration
of retrograde pulmonary vein flow has been shown to reflect the left ventricular end-
diastolic pressure. However, there are limitations to the evaluation of pulmonary vein
velocities including the difficulty in obtaining these samples in some patients.[61]

Tissue Doppler imaging (TDI) is a rather new imaging modality that is used to
assess myocardial velocities.[71] There is evidence to suggest that the early diastolic
myocardial velocity recorded at the mitral annulus is a preload-independent marker of
diastolic relaxation. The mitral E wave recorded by pulsed-wave Doppler
echocardiography is influenced by atrial pressure as well as by changes in the time
constant of relaxation. Therefore, the E wave relates poorly with left atrial pressure.
However, correction of the E wave velocity for its dependence on relaxation may
improve its relation with left atrial pressure. TDI studies of left ventricular inflow
support this belief. The ratio of E wave to early diastolic annular velocity (Ea)
demonstrates excellent correlation with mean pulmonary capillary wedge pressure
(PCWP) as has been shown in people with various clinical conditions.[71]
B. Diastolic dysfunction and HCM

Recent studies suggest that diastolic dysfunction is an important cause of congestive heart failure.[74-76] Many factors can lead to the development of impaired cardiac filling and abnormal relaxation. Such factors include myocardial ischemia, LV hypertrophy, cardiac effects of chronic renal failure, and advanced age. Other causes include systemic hypertension, aortic stenosis, and HCM.[77]

HCM is a primary myocardial disease; it is the 2nd most common idiopathic myocardial disease in people [15] and the most common cardiac disease in adult cats.[1] HCM is characterized by hypertrophy of a nondilated ventricle in the absence of identifiable cause.[1, 3, 5] Diastolic dysfunction is believed to be the primary pathophysiologic mechanism responsible for clinical signs.[4] In people, HCM is a genetic disease characterized by an autosomal dominant mode of inheritance.[6-8] HCM is heritable in Maine Coon Cats and familial forms of the disease are recognized in other breeds.[9-11] In cats it is often difficult to exclude all secondary causes of myocardial hypertrophy. In humans, HCM is usually characterized histologically by myocardial cellular disarray; however myocardial cellular disarray is seen in only about 25% of feline patients with myocardial hypertrophy. [2]

In cats, HCM is most commonly diagnosed in patients that are young adults or middle-aged, although it can be seen in cats of all ages. Different studies report the prevalence to be higher in male cats versus female cats (with male cats comprising 60-75%).[1, 78]
HCM is a major cause of morbidity and mortality in cats. It most often manifests clinically through signs of congestive heart failure, systemic thromboembolism and sudden death. HCM is often detected in a subclinical form when abnormalities detected on routine physical examination prompt echocardiographic examination.[1, 12] Feline HCM is characterized by left ventricular diastolic dysfunction and has a heterogeneous morphologic expression. Typically, it results in symmetric hypertrophy that involves the interventricular septum and posterior wall. Less often, the hypertrophy is asymmetrical or it may be segmental and involve only a portion of the interventricular septum or posterior wall.[1, 12] This differs from the HCM in humans where the hypertrophy is typically asymmetrical with the interventricular septum being more affected than the posterior wall.[15] In one study, asymmetrical hypertrophy was more common than symmetrical hypertrophy in canine patients with HCM; this is in contrast to other studies that show that canine patients, similar to affected cats, tend to have symmetrical hypertrophy.[15, 16]

Hypertrophy may decrease the size of the left ventricular cavity and increase myocardial stiffness. Fibrous replacement of myocardium or myocardial cell disorganization decreases ventricular compliance and impairs myocardial relaxation.[79] A decrease in early rapid filling results from reduced ventricular compliance and prolonged relaxation.[1] There is loss of the elastic recoil/suction forces in early diastole. This, and slowing of myocardial relaxation, leads to elevated early diastolic left ventricular pressure. The early diastolic left ventricular-left atrial pressure gradient decreases and there is increased reliance on atrial contraction for ventricular filling.[61]
Left atrial pressures rise and may result in pulmonary venous hypertension, pulmonary edema and possibly pleural effusion and right-sided heart enlargement.[79, 80]

The finding of mitral valve regurgitation is common in cats with HCM. This may result from distortion of the mitral valve apparatus due to the hypertrophy[1, 80] or may result from interference with normal mitral valve motion associated with anterior motion of the mitral valve during mid-systole:[1] this form of HCM is known as hypertrophic obstructive cardiomyopathy (HOCM).[80]

In human patients, HCM is histologically characterized by areas of myocardial fiber disarray that involves greater than 5% of the myocytes in the interventricular septum and free wall as well as an increase in interstitial fibrous tissue. It is these tissue changes that are most likely responsible for the increased ventricular chamber stiffness.[80] In feline patients, cardiac muscle cell disorganization occurs to a greater extent in the interventricular septum than in the posterior wall, however, its prevalence is less than that seen in humans. In one study, feline patients showed “swiss cheese” myocardial architecture, characterized by multiple small areas of cell-to-cell disarray.[16] Abnormal intramural coronary arteries are also commonly seen in feline as well as human patients with HCM. The arteries are usually characterized by thickened walls and narrowed lumens which are seen with equal frequency in cats as well as humans. Areas of interstitial fibrous tissue are much more common in humans than in cats, and abnormal coronary arteries are present more frequently in tissues with moderate-to-severe fibrosis. This suggests that the coronary artery abnormalities represent a form of “small vessel disease” and may contribute to myocardial ischemia and necrosis.[16, 81]
The diagnosis of HCM in feline patients may be suspected based on clinical signs but is confirmed by echocardiography. Electrocardiographic abnormalities include changes consistent with cardiac chamber enlargement, atrial fibrillation, ventricular tachycardia, ventricular premature complexes, and atrioventricular conduction abnormalities. The most commonly reported finding is an intraventricular conduction disturbance that results in a left anterior conduction block-type pattern.[1, 78] Arrhythmias are noted in approximately ¼-1/2 of affected cats.[1]

The echocardiographic diagnosis of HCM depends on the finding of abnormal left ventricular hypertrophy. It can be characterized by a variety of phenotypic patterns ranging from mild wall thickening involving only one component of the interventricular septum or posterior wall or diffuse hypertrophy that affects the free wall and septum equally or any pattern in between. The left ventricular cavity size is normal or decreased as a result of ventricular wall hypertrophy. Other echocardiographic findings may include left atrial and right atrial enlargement, hypertrophied papillary muscles, mild hypertrophy of the right ventricular wall and mild to moderate hypertrophy of the right ventricular outflow tract, normal to elevated fractional shortening, right and left ventricular dilation (seen late in the course of the disease) and pericardial effusion.[1] In cases of HOCM, there is dynamic left ventricular outflow tract obstruction. Systolic anterior motion of the mitral valve (SAM) is a common finding in patients with HCM. In one study of cats with HCM, 31 of 46 cats were identified as having SAM of the mitral valve; each of these cats also had color flow Doppler evidence of mitral valve regurgitation. The cats with SAM did not differ from the cats without SAM in regards to
left ventricular wall thickness.[4] In the same study, cats with HCM that died of heart failure had greater left ventricular thickness, larger left atria and tended to have the non-obstructive form of the disease.[4] The latter finding is in agreement with another retrospective study assessing the survival characteristics of cats with HCM and suggests that cats with the non-obstructive form and those with a large left atrium may have a less favorable prognosis.[4, 82]

Various echocardiographic methods have been used to assess diastolic function. PVI is used as an adjunct to mitral valve inflow in the assessment of diastolic function in both humans and cats. In human patients, the use of TDI has emerged as a new technique in the assessment of left ventricular diastolic function. While PVI velocities and mitral inflow patterns may suggest the presence of impaired left ventricular relaxation, a preload-independent non-invasive index of left ventricular relaxation, such as TDI may allow the evaluation of relaxation independent of loading conditions. TDI offers a means of assessing diastolic wall motion and is used to measure the velocity of myocardial wall motion in both systole and diastole.[61, 62, 83-85] Recent studies have shown that new Doppler techniques may be useful in characterizing diastolic function in cats.[13, 14]

III. Neurohormonal Activity and Cardiac Disease

In human medicine, there is a growing demand for the development of more specific and sensitive tests to identify early myocardial injury or myocardial dysfunction. This has led to investigation of many biochemical markers of cardiac dysfunction
Over the past few years, there has been a growing interest in neurohormonal activation in feline heart disease. Plasma endothelin-1 (ET-1), a potent vasoconstrictor peptide, is being investigated as a diagnostic test of cardiovascular disease in cats and the AA sequences of feline big ET-1 has been recently determined.[86] CTnI, a sarcomeric protein, has been shown to be a specific and sensitive marker for myocardial damage in humans and various other mammalian species.[87-90] Preliminary normal ranges of plasma cTnI in normal cats has been determined.[91] Two separate studies investigating the plasma cTnI levels in cats with HCM and in normal cats have shown that the plasma concentration of cTnI is significantly higher in cats with HCM than in normal cats.[92, 93]

The plasma concentration of BNP has gained widespread acceptance as a diagnostic and prognostic marker for cardiac dysfunction and congestive heart failure in human patients.[17-19] BNP sequence is species-specific and has recently been determined in cats.[94] As well, in multiple studies, plasma levels of ANP have been found to be markedly elevated in human patients with heart diseases such as aortic or mitral stenosis, [20-22] dilated cardiomyopathy [20, 22] or heart disease as a result of tachycardia.[23, 24] In addition, several studies have sought to assess the importance of ANP in the diagnosis and/or prognosis of human patients with HCM.[25-30]

HCM is characterized by delayed myocardial relaxation and abnormal chamber stiffness that results in diastolic dysfunction. Impaired ventricular relaxation leads to a
compensatory increase in the contribution of atrial systole to left ventricular filling and results in elevated end-diastolic filling pressures.[15]

ANP is predominantly produced and secreted in response to atrial volume or pressure overload. Plasma [ANP] is increased in patients with congestive heart failure and correlates well with left ventricular end-diastolic and atrial pressures.[31-34] Therefore, the relationship between plasma [ANP] and the severity of heart failure likely reflects, to some extent, the higher atrial pressure associated with more severe heart failure.[30]

In a study by Lang et al., patients with diastolic dysfunction had a marked increase in [ANP] in the absence of significant left ventricular hypertrophy or systolic dysfunction. The presence of enlarged left atria compared with controls implied that the diastolic dysfunction was associated with high left atrial pressures and this led to elevated [ANP]. In the same study, BNP concentration was associated with the degree of diastolic dysfunction, presumably as a result of elevated ventricular wall stress due to the higher left ventricular end-diastolic pressure.[95]

Over the past decade, many studies have assessed the role of ANP as a diagnostic/prognostic tool in diastolic dysfunction. While most studies agree that plasma [ANP] is increased in congestive heart failure, studies in patients with HCM differ in their findings regarding the association of ANP with echocardiographically-derived parameters of diastolic function or left ventricular/left atrial dimensions. The study by Derchi et al. showed a positive correlation between interventricular septum thickness and [ANP], as well as atrial size and [ANP].[25] The study by Fahy et al., found elevated
[ANP] in patients with HCM but did not find any correlation between echocardiographically-derived parameters of left ventricular structure or diastolic function. [27] The study by Briguori et al showed a correlation between [ANP] and left atrial fractional shortening.[26] Lastly, in the study by Kitaoka et al, the prognostic significance of plasma [ANP] was determined to be the most important prognostic factor for all adverse cardiovascular events including sudden cardiac death, heart failure, peripheral embolism and ischemic stroke. [35]

The prognosis associated with feline HCM is variable and the factors that determine clinical outcome are incompletely described. As well, there is a lack of information regarding neuroendocrine activity in cats. The recognition of the relationship between neurohormones, cardiac hemodynamics and structural abnormalities has raised the prospect of using natriuretic peptides as indicators of cardiac dysfunction. Knowledge of plasma [ANP] in affected feline patients may provide useful information regarding cardiac structure and function in several situations.

In this study, we measured plasma [Nt-proANP] of 17 feline patients with HCM. Nineteen student owned cats served as controls. The relationships between plasma [Nt-proANP] and echocardiographic variables were evaluated. Specifically, the relationship between plasma [Nt-proANP] and left ventricular internal diastolic dimension, measurements of ventricular wall thickness, left ventricular fractional shortening, left atrial fractional shortening, mitral valve inflow velocity and the rate of deceleration of left ventricular diastolic filling was determined. Additionally, the clinical relevance of
variables such as gender, age, heart rate and body weight on plasma [Nt-proANP] was evaluated.

This preliminary investigation will elucidate the clinical relevance of plasma [Nt-proANP] in feline patients with HCM and will form the basis for studies that will define the prognostic relevance of plasma [Nt-proANP] in patients with this disorder.
CHAPTER II
PLASMA N-TERMINAL PROATRIAL NATRIURETIC PEPTIDE
CONCENTRATION IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY

by
Heidi N. MacLean
Jonathan A. Abbott, Chair
(ABSTRACT)

Objective: We sought to determine N-terminal proatrial natriuretic peptide concentration [Nt-proANP] in plasma from cats with hypertrophic cardiomyopathy (HCM).

Secondarily, we wished to evaluate the relationship between [Nt-proANP] and echocardiographic variables.

Methods: Venous blood samples were obtained from seventeen cats with HCM and from nineteen healthy cats. Plasma [Nt-proANP] was determined using an ELISA assay. The relationship between plasma [Nt-proANP] and M-mode, 2-dimensional and Doppler echocardiographic variables was evaluated. Cats that were hyperthyroid or had evidence of renal disease were excluded from the study.

Results: The mean plasma [Nt-proANP] was higher in cats with HCM (3.81 +/- 1.23 pmol/l) than in control cats (3.08 +/- 1.41 pmol/l); however, this difference was not statistically significant (p=0.17). There was a significant correlation between plasma [Nt-proANP] and left ventricular posterior wall thickness (r = 0.42; p=0.01). Additionally, plasma [Nt-proANP] was correlated with left atrial size (r = 0.35; p=0.03).
A linear regression model was developed to further explore these relationships. LAs2D and LVPWd had an interactive effect on plasma [Nt-proANP] ($R^2 = 0.2737; p= 0.02$). There was no correlation between any other echocardiographic variable and plasma [Nt-proANP]. There was no correlation between plasma [Nt-proANP] and heart rate (HR), body-weight, or age.

**Conclusions:** Cats with HCM do not have significantly higher plasma [Nt-proANP] than normal cats. There was a significant linear relationship between [Nt-proANP] and LAs2D, LVPWd and the model that described their interaction.
B. Introduction

HCM is the most prevalent cardiac disease in adult cats.[1] It is characterized by hypertrophy of a nondilated ventricle in the absence of identifiable cause.[2-4] Feline HCM is characterized by left ventricular diastolic dysfunction[13, 14] and has a heterogenous morphologic expression[2].

ANP is a polypeptide that has natriuretic, diuretic and vasodilatory properties and contributes to the regulation of blood pressure and fluid homeostasis. ANP is predominantly produced and secreted by the atria in response to volume or pressure overload. Plasma [ANP] is increased in patients with congestive heart failure and it correlates well with left ventricular end-diastolic pressures and atrial pressures.[31-34] Therefore, association between plasma [ANP] and the severity of heart failure, may reflect, to some extent, the higher atrial pressure associated with more severe heart failure.[30]

Recently, the prognostic role of plasma [ANP] has been investigated in human patients with HCM. Patients with high plasma [ANP] had a poorer prognosis than those patients with low plasma [ANP]. As well, plasma [ANP] was the most important prognostic factor for all adverse cardiovascular events including sudden cardiac death, heart failure, peripheral embolism and ischemic stroke.[35] Nt-proANP is released into the circulation in equal amounts as C-terminal ANP (mature ANP). However, the plasma half-life of Nt-proANP is 8 times as long as C-terminal ANP and therefore, the plasma concentration of Nt-proANP is about 10 times higher than that of C-terminal ANP.[46]
In humans, it has been shown that the variation in Nt-proANP is less than C-terminal ANP and therefore, better suited for diagnostic or prognostic use.[96]

Unfortunately, there is a paucity of information regarding neuroendocrine activation in cats with heart disease. Furthermore, the clinical outcome in feline patients with HCM is difficult to predict. The proposed investigation will test the hypothesis that plasma [Nt-proANP] in feline patients with HCM exceeds that in healthy cats. The primary study objective was to determine plasma [Nt-proANP] in cats with HCM and in a control sample of healthy cats. Secondarily, we wished to evaluate the relationship between [Nt-proANP] and echocardiographic variables. This study will help elucidate the clinical importance of plasma [Nt-proANP] in feline HCM and will form the basis for studies that will define the prognostic relevance of plasma [Nt-proANP] in patients with this disorder.

C. Materials and Methods

Study sample

Feline patients with hypertrophic cardiomyopathy that were presented to the VMRCVM Clinical Cardiology Service between October 2002 and November 2003 were included in the study. The control sample consisted of echocardiographically normal cats that presented to the VMRCVM Cardiology Service during the same period and healthy cats that were recruited from pet-owning veterinary students and house officers of the VMRCVM. This study was approved by the Virginia Tech Animal Care Committee.
Written consent was obtained from the pet-owner prior to inclusion in the study. All cats were subject to physical examination and echocardiographic study. Indirect systemic arterial blood pressure was obtained from all cats. In addition to determination of plasma [Nt-proANP], further biochemical or endocrine testing was performed as described below.

Cats were included in the study group if the end-diastolic thickness of the interventricular septum (IVSd) or left ventricular posterior wall (LVPWd) obtained by M-mode echocardiography was \( \geq 6\) mm provided the end-diastolic LV chamber dimension (LVIDd) was \( \leq 18\) mm. Congestive heart failure was diagnosed when the presence of pleural effusion or pulmonary edema was radiographically evident. Cats that were receiving cardiovascular medications (other than furosemide) or that had a history of chronic renal failure, systemic hypertension or hyperthyroidism, were excluded from the study.

Plasma collection

After obtaining informed, written owner consent, each cat was confined alone to a dark, quiet room for 30 minutes. After the acclimation period, the subject was placed in lateral or sternal recumbency and approximately 6-8mls of blood were obtained from the right or left jugular vein. A 2ml aliquot of blood was placed in a tube containing EDTA for blood urea nitrogen (BUN) and creatinine determination. \(^a\) If the cat was older than 6 years of age, 2mls of blood were placed in a glass tube for T4 analysis. \(^b\) After venipuncture, the subjects were returned to the quiet environment for approximately 10
minutes. Systolic arterial blood pressure was estimated using a Doppler flowmeter and manometric cuff; 3 measurements were recorded and averaged. Cats in which systolic blood pressure exceeded 180mmHg were excluded from the study. Cats with a BUN >34mg/dL, serum creatinine concentration >1.9mg/dL, or serum T4 concentration >4.0ug/dL as determined on the day of the study, were also excluded.

**ProANP assay preparation**

4.5 mls of blood were placed in pre-chilled 5-ml polypropylene tubes that contained 24ul of EDTA and 0.35ml of aprotinin for later determination of [Nt-proANP]. The samples were placed in an ice bath until they were centrifuged (within 15 minutes of collection). The samples were centrifuged at 1500rpm for 15 minutes at 0° C. The supernatant was then collected and placed in a 5-ml polypropylene tube and stored at –70° C until [Nt-proANP] determination.

**ProANP (1-98) analysis**

[Nt-proANP] was determined using a commercially available sandwich enzyme immunoassay (EIA) kit specific for Nt-proANP (1-98) from BIOMEDICA®. All samples were analyzed on the same day (December 9, 2003) using the same EIA kit. This BIOMEDICA assay is intended for the measurement of human [Nt-proANP] from biological fluids. This assay uses two polyclonal antibodies to amino acids 10-19 and 85-90 of Nt-proANP, which are identical between human and feline ANP.[46] Precision
studies done by the kit manufacturer showed an interassay coefficient of variance (CV) of 6.3% at a mean concentration of 427fmol/ml and an intrassay CV, based on five replicates, of 6.6% at a mean concentration of 436fmol/ml. The range of the standard curve is 0 to 5000fmol/ml. The detection limit of the assay, as defined as 3 standard deviations (SD) above the mean concentration of the zero standard when run as unknown, is 50fmol/ml. In humans, crossreactions with other ANP, BNP and CNP molecules is <1%.d

Echocardiographic examination

Echocardiographic examination was performed using a Vingmed System-FiVe® sonograph equipped with a 7.5mHz transducer. All echocardiographic examinations were performed by one investigator (JAA). Echocardiography was performed within 20 minutes of phlebotomy. Each subject was conscious and manually restrained. Two-dimensional, M-mode, spectral and color Doppler echocardiography were performed and digitally stored for later analysis. Standard images were acquired as previously described.[97-99] The following variables were obtained: septal and left ventricular posterior wall thickness during systole (IVSs and LVPWs respectively) and diastole (IVSd and LVPWd respectively), left atrial dimension (obtained from a standard right parasternal M-mode image [LADs], and from a 2-dimensional right parasternal image that included the left ventricle, left atrium and aorta in long axis [LAs2D]), minimal and maximal left atrial dimensions obtained from M-mode image (Lamin and Lamax, respectively), isovolumic relaxation time (IVRT), and peak velocity of early diastolic
filling (MVE) and late diastolic filling (MVA), rate of deceleration of mitral valve inflow 
(MVE/DT) and presence of absence of systolic anterior motion of the mitral valve 
(SAM). From these data, the following were derived: an index of ventricular 
hypertrophy (LVPWd + IVSd/ LVIDd), an index of left atrial size (LADs/LVIDd), left 
ventricular and left atrial fractional shortening and LADs/Ao.

**Statistical Analysis**

All echocardiographic measurements were performed by averaging three, usually 
consecutive, cardiac cycles. Data were expressed as the mean +/- 1 standard deviation 
unless otherwise noted. All statistical analyses were performed using a commercial 
software program. Student’s t-test was used to compare the difference between [Nt-
proANP] in cats with HCM and control cats. Pearson’s product-moment correlation 
coefficient was used to determine the strength of relationship between Nt-proANP and 
the various echocardiographic variables. Correlation coefficients were considered 
significantly different from zero if p<0.05. For echocardiographic variables that were 
significantly correlated with plasma [Nt-proANP], multiple linear regression was used to 
explore the interactive relationship between the variables and [Nt-proANP]. Residual 
plots were evaluated to confirm that the assumptions required for regression analysis 
were met.
D. Results

Seventeen cats (14 males and 3 females) with HCM were included in the study. They ranged in age from 1-12 years (mean 6.1 +/- 3.0 years) and body weights ranged from 3.9 to 7.1kg (mean 5.4 +/- 1.0 kg). There were 16 mixed breed cats, and 1 Persian cat. Two cats were receiving furosemide for the treatment of congestive heart failure. The presence of congestive heart failure was diagnosed when the presence of pleural effusion or pulmonary edema was noted on thoracic radiographs. The control sample consisted of 19 cats (11 males and 8 females). They ranged in age from 6 months to 9 years (mean 5.0 +/- 2.7 years) and body weights ranged from 1.6 to 6.8kg (mean 5.1 +/- 1.2 kg). Breeds included 15 mixed breed cats, 1 himalayan, 1 persian and 1 siamese.

Measurements of LADs(min) and LADs(max) were not obtained from one cat with HCM and MVE/DT, MVE and MVA, IVRT and HR were not obtained from four cats with HCM due to a technical problem with the optical disk on which the echocardiographic images were stored. Late diastolic inflow velocities were not recorded in 13 HCM cats and 8 control cats due to the lack of MVA on spectral Doppler.

The mean plasma [Nt-proANP] was higher in cats with HCM (3.81 +/- 1.23 pmol/l) than in control cats (3.08 +/- 1.41 pmol/l); however, this difference was not statistically significant (p=0.17) (Figure 1). There was a statistically significant correlation between the plasma [Nt-proANP] and LVPWd (r = 0.42; p=0.01)( Figure 2). Additionally, plasma [Nt-proANP] was correlated with LAs2D (r = 0.35; p=0.03) (figure 3). LAs2D and LVPWd had an interactive effect on plasma [Nt-proANP] and this relationship was included in the model (Figure 4). The variability in the data were best
described by the following equation: plasma [Nt-proANP] = 12031 – 7779.76 (LAs2D) - 15957(LVPWd) + 13823 (LAs2D x LVPWd) \((R^2 = 0.2737; p= 0.02)\).

There was no correlation between any of the other echocardiographic variables and plasma [Nt-proANP]. There was no correlation between plasma [Nt-proANP] and heart rate, body-weight, or age.

E. Discussion

Plasma [Nt-proANP] was not significantly greater in cats with HCM than in control cats. However, plasma [Nt-proANP] was correlated with both LVPWd and LAs2D, and the variability in [Nt-proANP] was best explained by a linear model which included LVPWd, LAs2D and their interaction.

This result may best be explained by the release mechanism and secretion of ANP. ANP is believed to be regulated by myocardial wall stretch, or by wall stress as determined by the Law of Laplace.[101] Feline HCM results in diastolic dysfunction and patients with this disease have impaired myocardial relaxation and reduced chamber compliance.[1, 102] Replacement of myocardium with fibrous tissue or myocardial cell disorganization impairs myocardial relaxation and decreases ventricular compliance.[79] Loss of elastic recoil/suction forces in early diastole and a decrease in early rapid filling results from prolonged relaxation.[1] This, and the slowing of myocardial relaxation, leads to elevated left ventricular filling pressures. The left ventricular-left atrial pressure gradient decreases and there is an increased reliance on atrial contraction for further ventricular filling; as a result, left atrial pressures rise.[61] Therefore, increased left atrial
pressures and increased left ventricular myocardial wall stress may result in elevated plasma [Nt-proANP].

In this study, Nt-proANP was measured rather than C-terminal ANP. In humans, it has been shown that the variation in Nt-proANP is less than C-terminal ANP and therefore, better suited for diagnostic or prognostic use. [96] Nt-proANP is released into the circulation in equal amounts as C-terminal ANP. However, the plasma half-life of Nt-proANP is 8 times longer than C-terminal ANP and therefore, the plasma concentration of Nt-proANP is about 10 times higher than that of C-terminal ANP. As well, Nt-proANP is more stable in vitro than C-terminal ANP as Nt-proANP appears to be stable in EDTA-anticoagulated blood for up to 6 hours at room temperature.[46] Lastly, in humans there is convincing evidence that Nt-proANP is superior to C-terminal ANP as a prognostic indicator of mortality after myocardial infarction and the data suggested that the risk of death is closely correlated to [Nt-proANP] in people with congestive heart failure.[100]

A study that evaluated cardiac expression of ANP in cats with various cardiomyopathies demonstrated that cats with HCM may have ventricular expression of ANP rRNA. Variable amounts of ANP have also been found in dogs and humans with hypertrophied hearts.[29, 103] However, an investigation of ANP and BNP gene expression in normal cats and cats with HCM showed that ANP immunoreactivity was diffuse throughout the atria but absent within the ventricles.[41] In a human study of HCM, plasma [ANP] was associated with interventricular septal thickness, left ventricular posterior free wall thickness and left atrial size. They speculated that atrial
stretch, ventricular hypertrophy and increased filling pressures may be stimuli for ANP release. A recent study in humans with HCM showed that ventricular ANP gene expression occurred in response to disease specific changes including myocardial fiber disarray, hypertrophy of myocytes and fibrosis rather than as an adaptive response to hemodynamic overload.[29] In our study, it is possible that the cats with HCM had elevated left atrial pressures leading to the elevation of plasma [Nt-proANP]. However, ventricular expression of [Nt-proANP] cannot be discounted.

There is another possible mechanism for the association between plasma [Nt-proANP] left ventricular wall thickness and atrial size. Cats with HCM have intramural coronary artery disease. In a study by Liu et al[16], abnormal coronary arteries were evident in cats with HCM, but were present more commonly in areas of moderate-to-severe myocardial fibrosis; therefore, this small vessel disease may contribute to myocardial ischemia and necrosis. The presence of myocardial hypertrophy without adequate increases in coronary artery reserve in cats with HCM may also lead to myocardial ischemia.[104] Humans with HCM have abnormal coronary flow dynamics, decreased coronary artery vasodilatory reserve and systolic compression of the septal perforator arteries; together, these abnormalities may contribute to myocardial ischemia and necrosis.[93, 105, 106] Larsen et al [107] demonstrated elevated [ANP] in cats with acute myocardial ischemia and the plasma [ANP] had a positive linear correlation to left ventricular end diastolic pressures. Elevated plasma [ANP] has also been demonstrated in human patients with myocardial infarctions where plasma [ANP] was shown to increase with infarct size.[108]
In other human studies, plasma [ANP] was elevated in patients with HCM as well as those with CHF due to other cardiac diseases.[109, 110] The cause of high [ANP] observed in people with HCM has not been established. Some investigators [25, 111] have described a relationship between atrial size and plasma [ANP], although this finding is not universal.[27] Briguori et al.,[26] reported a strong inverse correlation between plasma [ANP] and atrial fractional shortening. Atrial fractional shortening is an echocardiographic parameter of diastolic function that is correlated with LV end-diastolic pressure.[112] In our study, we did not see a significant correlation between these two variables. As well, there was no correlation between plasma [Nt-proANP] and LADs. These findings may reflect the insensitivity of M-mode echocardiographic examination in the detection of left atrial enlargement.

To the author’s knowledge, this is the first published report of plasma [Nt-proANP] in feline HCM. However, there has been recent investigation into this topic by others. In one study, plasma [Nt-proANP] was analyzed in cats with various myocardial diseases including HCM, restrictive cardiomyopathy, and unclassified cardiomyopathy, with and without CHF or systemic thromboembolism. The authors found elevated [Nt-proANP] in cats with cardiomyopathy with and without CHF or systemic thromboembolism. The cats were grouped together into control or myocardial disease, and therefore, it is unclear whether or not cats with HCM had elevated plasma [Nt-proANP]. Another study investigating [ANP] in normal cats and in cats with HCM and/or hyperthyroidism was performed. Using a commercially available radioimmunoassay
(RIA), validated for use in the cat, they found that the plasma [ANP] was significantly increased in cats with HCM compared to normal cats.\(^1\)

The results of the present study were different from the latter study in that there was no significant difference in plasma [Nt-proANP] between cats with HCM and normal cats. This may be due to several factors. First, we analyzed plasma [Nt-proANP], not [ANP], and therefore, this difference alone may account for the different results. Secondly, we measured the plasma [Nt-proANP] using an EIA test rather than a RIA and therefore comparison between the studies is difficult.

Recently, investigators measured [ANP] in kidneys of cats with HCM. Renal [ANP] was lower in control cats and in cats with HCM than in normal humans. Therefore, renal [ANP] may function locally in the kidneys since the mRNA and tissue concentrations were too low to contribute to plasma concentrations. However, cats with HCM may have increased activity of renal [ANP].\(^1\) None of these studies assessed the correlation between plasma [Nt-proANP] and echocardiographic variables.

Over the past decade, many studies have evaluated the role of ANP as a diagnostic/prognostic tool in diastolic dysfunction. While most studies agree that plasma [ANP] is increased in CHF, studies in patients with HCM differ in their findings regarding the association of [ANP] with echocardiographically-derived indices of diastolic function or left ventricular/left atrial dimensions. The study by Derchi et al. [25] showed a positive correlation between interventricular septal thickness and ANP concentration, as well as atrial size and [ANP]. The study by Fahy et al. [27] found elevated [ANP] in the patients with HCM but did not find any correlation between
echocardiographically-derived parameters of left ventricular structure or diastolic function. In the study by Briguori et al showed a correlation between [ANP] and left atrial fractional shortening.[26] Kitaoka et al[35] found that plasma [ANP] was an independent risk factor for adverse cardiovascular events including sudden cardiac death, heart failure, peripheral embolism and ischemic stroke.

This study was limited by a small sample size. A significant difference in plasma [Nt-proANP] between HCM cats and control cats might have been detected if more cats had been enrolled in the study. Additionally, left ventricular function was assessed non-invasively. There are no universally accepted echocardiographic criteria that define diastolic dysfunction. Echocardiographic parameters used to diagnose diastolic dysfunction tend to be preload and afterload dependent making them difficult to use in clinical practice.

We detected a linear relationship between left atrial size, left ventricular posterior wall thickness and an interaction of these variables with plasma [Nt-proANP]. In humans, plasma ANP is an independent risk factor for adverse cardiovascular events including sudden cardiac death, heart failure, peripheral embolism and ischemic stroke.[35] It is our hope that the information gained from this study can be used in the future to help form the basis for other studies that will define the prognostic relevance of [Nt-proANP] in cats with HCM.
CHAPTER III

Conclusions

In this study, a group of cats with HCM did not have significantly higher mean plasma [Nt-proANP] than normal cats. In cats, plasma [Nt-proANP] was correlated with left atrial systolic dimension. Although not universally accepted, this finding is in accord with the human literature in which atrial stretch and elevated left atrial pressures are believed to be responsible for the elevation in plasma [Nt-proANP] in patients with HCM and enlarged left atria.

In cats, plasma [Nt-proANP] was correlated with left ventricular free wall thickness. Left atrial systolic dimension and left ventricular free wall diastolic diameter interacted in their effect on [Nt-proANP]. In humans, plasma [ANP] is believed to be an important prognostic factor for all adverse cardiovascular events. It is our hope that the information gained from this study can be used in the future to help form the basis for other studies that will define the prognostic relevance of [Nt-proANP] in cats with HCM.
FOOTNOTES

a. Clinical Pathology Service, Veterinary Teaching Hospital, VMRCVM

b. Antech Laboratories, Atlanta, GA

c. Park’s Doppler Flow Detector, Aloha, OR

d. American Laboratory Products Company, Windham, NH.

e. General Electric Medical Systems

f. SAS, Version 8.02, SAS Institute Inc. Cary, NC


REFERENCES


Figure 1. Box and whisker plot of plasma [Nt-proANP] in 17 cats with hypertrophic cardiomyopathy (HCM) and 19 control cats. The boxes represent the interquartile range (IQR). The lines crossing the shaded boxes represent medians and the solid circles represent means. The whiskers extend to the most extreme observation that is less than 1.5 X IQR from the upper and lower limits of the IQR. Observations more than 1.5 X IQR from the upper and lower limits of the IQR are indicated by asterisks.
Figure 2. A scatterplot that demonstrates the correlation between plasma Nt-proANP concentration [Nt-proANP] and left ventricular posterior wall diastolic thickness (LVPWd) as measured from a long-axis right parasternal transducer position in 17 cats with HCM and 19 control cats ($r = 0.42; p=0.01$).
Figure 3. A scatterplot that demonstrates the correlation between plasma Nt-proANP concentration [Nt-proANP] and left atrial systolic dimension (LAs2D) as measured from a long-axis right parasternal transducer position in 17 cats with HCM and 19 control cats (r = 0.35; p=0.03).
Figure 4. Regression model of the interactive relationship of LAs2D (left atrial size, during systole, as measured by 2-dimensional echocardiography from a right parasternal transducer position - measured in centimeters) and LVPWd (left ventricular posterior wall diastolic thickness, as measured by 2-dimensional echocardiography from a right parasternal transducer position - measured in centimeters) with plasma [Nt-proANP] in 17 cats with HCM and 19 control cats.
VITA

Heidi N. MacLean

PERSONAL INFORMATION

Date of birth: April 17, 1973
Home town: Charlottetown, Prince Edward Island, Canada

EDUCATION

<table>
<thead>
<tr>
<th>Date</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2004</td>
<td>Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA</td>
</tr>
<tr>
<td></td>
<td>Master of Science in Veterinary Clinical Sciences</td>
</tr>
<tr>
<td>July 2004</td>
<td>Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA</td>
</tr>
<tr>
<td></td>
<td>Residency in Veterinary Cardiology</td>
</tr>
<tr>
<td>July 2001</td>
<td>Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA</td>
</tr>
<tr>
<td></td>
<td>Internship in Small Animal Medicine and Surgery</td>
</tr>
<tr>
<td>May 2000</td>
<td>Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada</td>
</tr>
<tr>
<td></td>
<td>Doctor of Veterinary Medicine</td>
</tr>
<tr>
<td>1991-1995</td>
<td>University of Prince Edward Island, Charlottetown, PEI, Canada</td>
</tr>
<tr>
<td></td>
<td>Bachelor of Science in Biology, First Class Honours</td>
</tr>
</tbody>
</table>

PROFESSIONAL EXPERIENCE

<table>
<thead>
<tr>
<th>Date</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2001 – July 2004</td>
<td>Residency in Veterinary Cardiology, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA</td>
</tr>
<tr>
<td>June 2000 – July 2001</td>
<td>Small Animal Medicine and Surgery Internship, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA</td>
</tr>
<tr>
<td>April - June 2000</td>
<td>Student Veterinary Clinical Position, Small Animal Hospital at the Atlantic Veterinary College, UPEI</td>
</tr>
<tr>
<td>September 1999</td>
<td>Emergency Medicine Externship, Tufts University School of Veterinary Medicine, North Grafton, MA</td>
</tr>
<tr>
<td>June - Sept 1999</td>
<td>Student Veterinary Clinical Position, Small Animal Hospital at the Atlantic Veterinary College (volunteer), UPEI</td>
</tr>
</tbody>
</table>

AWARDS

Atlantic Veterinary College

<table>
<thead>
<tr>
<th>Year</th>
<th>Award Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Bimed-MTC Animal Health Inc. Award for clinical proficiency in small animal and large animal medicine and surgery</td>
</tr>
<tr>
<td></td>
<td>3rd highest aggregate overall in the 4 year veterinary program</td>
</tr>
<tr>
<td>1998-1999</td>
<td>5th highest aggregate in 3rd year veterinary medicine</td>
</tr>
<tr>
<td>1997-1998</td>
<td>G. Murray &amp; Hazel Hagerman Scholarship (Gift of Verna Blanchard) for student standing 2nd highest aggregate in 2nd year veterinary medicine</td>
</tr>
</tbody>
</table>
- Ayerst Veterinary Laboratories Award for Proficiency in Veterinary Bacteriology and Mycology
- The St. Andrews Society Bursary

1996-1997
- G. Murray & Hazel Hagerman Scholarship (Gift of Verna Blanchard) for student standing 1st highest aggregate in 1st year veterinary medicine
- Douglas W. Ehresmann Memorial Award for Proficiency in Virology
- The Veterinary Microscopic Anatomy Award for highest aggregate in Microscopic Anatomy
- The Veterinary Macroscopic Anatomy Award for highest aggregate in Macroscopic Anatomy
- The St. Andrews Society Bursary

University of Prince Edward Island (Biology Degree)
Graduation 1995
- Full tuition scholarship maintained through the 4 year bachelor program
- Industry, Science and Technology Canada: $2,500/year. Canada Scholarship Award maintained through the 4 year bachelor program
- Diagnostic Chemicals Ltd. Award for the 2nd highest aggregate in the Science Degree Program at UPEI

RESEARCH EXPERIENCE/PUBLICATIONS

July 2001-July 2004
Masters of Science in Veterinary Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA
Plasma atrial natriuretic peptide concentration in cats with moderate or severe hypertrophic cardiomyopathy.

January 2004
Scientific Publication, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

July 2002
Scientific Publication, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

June - Sept 1998
Research Assistant, Atlantic Veterinary College, Charlottetown, PEI
Studied the prevalence of Johne’s Dz, Bovine Viral Diarrhea Disease, Staphlococcus aureus mastitis, Neospora spp., and GI parasites in Holsteins within the Maritime provinces.

July - August 1997
Research Assistant, Atlantic Veterinary College, Charlottetown, PEI
Studied nutritional requirements and mastitis in Holstein cattle.

June - Sept 1996
Research Assistant, Atlantic Veterinary College, Charlottetown, PEI
Studied the concentration of insecticides and pesticides in ground water on PEI.

June - Sept 1995
Research Assistant, University of Prince Edward Island, Charlottetown, PEI
Surveyed numerous waterways and impoundments and analyzed the effects of pH, specific gravity, nitrates, temperature and death on fish health.

PRESENTATIONS

November 2003
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

A clinical approach to left ventricular diastolic function.

January 2003
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

The role of Beta-blockers in heart failure.

August 2002
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Plasma atrial natriuretic peptide concentration in cats with moderate or severe hypertrophic cardiomyopathy

June 2002
Poster presentation at the 20th annual ACVIM forum, Dallas, TX.

Comparison of Doppler-derived peak aortic velocities obtained from subcostal and apical transducer sites in healthy dogs.

January 2002
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Comparison of Doppler-derived peak aortic velocities obtained from subcostal and apical transducer sites in healthy dogs.

September 2001
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

March 2001
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Balloon dilation of double-chambered right ventricle in a cat.

November 2000
Graduate Seminar, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Paradoxical central vestibular disease in a three year old labrador retriever

February 2000
Clinical Conference (scientific presentation), Atlantic Veterinary College, Charlottetown, PE, Canada

Paradoxical central vestibular disease in a three year old labrador retriever

PROFESSIONAL ORGANIZATIONS

American Veterinary Medical Association
Canadian Veterinary Medical Association