SERIAL ECHOCARDIOGRAPHIC EVALUATION OF 22 CLOSELY RELATED GREAT DANES

A Thesis

by

MICHAEL RYAN FARMER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Veterinary Medical Sciences

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ABSTRACT

Serial Echocardiographic Evaluation of 22 Closely Related Great Danes.

(May 2009)

Michael Ryan Farmer, BS, Texas A&M University

Chair of Advisory Committee: Dr. Theresa W. Fossum

Objectives: The purpose of this study was to investigate a family of Great Danes with known dilated cardiomyopathy (DCM) using serial echocardiographic evaluation.

Animals, Materials, & Methods: Twenty-two dogs were included in this study. They were split into two groups, clinically normal and those with DCM. The dogs were scanned using 2D and M-mode echocardiography every thirty to sixty days beginning at approximately14-20 days of age. Data were collected and analyzed using generalized additive mixed regression, linear regression, and non-linear regression.

Results: All dogs demonstrated progressive echocardiographic changes. The Great Danes with DCM showed several echocardiographic differences when compared to the normal dogs. They included differences in left ventricular diameter, left atrial diameter, interventricular septal thickness, ejection fraction, and fractional shortening.

Conclusions: The present study shows that progressive echocardiographic changes occur in both clinically normal Great Danes and those with DCM as they mature. Additionally, the two groups differed with regards to left ventricular diameter,

left atrial diameter, interventricular septal thickness, ejection fraction, and fractional shortening.

DEDICATION

To my family and friends for their guidance and support.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Fossum, and my committee members, Dr. Miller, Dr. Gordon, and Dr. Clubb, for their guidance and support throughout the course of my study. The knowledge, experience, and friendships gained are invaluable. I would also like to thank Dr. Simon Sheather for his help with the statistical analysis.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience.

Finally, thanks to my family for their encouragement and to my wife and son for their patience and love.

NOMENCLATURE

Ao	Aortic Root
DCM	Dilated Cardiomyopathy
ECG	Electrocardiogram
HCM	Hypertrophic Cardiomyopathy
IVSd	Interventricular Septal Thickness at End-Diastole
IVSs	Interventricular Septal Thickness at End-Systole
LA/Ao	Left Atrium to Aortic Ratio
LADs	Left Atrial Diameter at End-Systole
LVDd	Left Ventricular Diameter at End-Diastole
LVDs	Left Ventricular Diameter at End-Systole
LVEF	Left Ventricular Ejection Fraction
LVPWd	Left Ventricular Posterior Wall Dimensions at End-Diastole
LVPWs	Left Ventricular Posterior Wall Thickness at End-Systole
% FS	Percent Fractional Shortening
RCM	Restrictive Cardiomyopathy
WHO	World Health Organization

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CHAPTER I

INTRODUCTION

Cardiomyopathy is a term first used in 1957 by Brigden to describe isolated, non-coronary myocardial disease.[1] Several years later, investigators described the classification of cardiomyopathy into three major categories; dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and restrictive cardiomyopathy (RCM).[2-6] In 1995 the World Health Organization (WHO) defined cardiomyopathy as a disease of myocardial dysfunction with four major classifications; DCM, HCM, RCM, and arrythmogenic right ventricular cardiomyopathy.[2,7] Today, the WHO classification is the most widely accepted.[2]

Dilated cardiomyopathy (DCM) is considered one of the most commonly acquired cardiac disorders in the dog.[8] It affects many medium and large dogs, with the Great Dane being reported as one of the most commonly affected breeds.[8-10] There are several known causes of DCM in the dog that include, genetic factors, tachycardia, drugs or toxins, and nutritional deficiencies.[2,8,11] The high prevalence of DCM in specific breeds has spurred genetic research, but to date no causative mutations have been identified.[12]

Echocardiography is an important diagnostic tool frequently used in veterinary cardiology to both initially diagnose and serially evaluate heart disease.[13] It is currently

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This thesis follows the style of The Journal of Veterinary Cardiology.

one of the most widely used imaging techniques available for the confirmation of cardiac disease, such as DCM.[14,15] Typical echocardiographic features of DCM include but are not limited to dilation of the left ventricle and atrium, decreased left ventricular ejection fraction (LVEF), and decreased percent fractional shortening (% FS).[11,15]

CHAPTER II

ANIMALS, MATERIALS, AND METHODS

This study examined twenty-two dogs from a local colony of Great Danes (11 males and 11 females) with a predisposition to early-age onset DCM (Fig. 1). The animals were evaluated echocardiographically every thirty to sixty days beginning at approximately 14-21 days of age, or as directed by the veterinary cardiologist. All M-mode and 2D echocardiographic examinations were performed using a 3 MHz transducer on a GE Vivid 5 or 7 echocardiograph, while simultaneously recording a standard lead-II electrocardiograph (ECG). Each animal was positioned in right and left lateral recumbency, and imaged from below through a notch in the table.[16] The following M-mode measurements were collected: left ventricular diameter at end-diastole (LVDd), left ventricular diameter at end-systole (LVDs), left ventricular posterior wall thickness at end-diastole (LVPWd), left ventricular posterior wall thickness at end-systole (LVPWs), interventricular septal thickness at end-diastole (IVSd), interventricular septal thickness at end-systole (IVSs), aortic root (Ao), left atrial diameter at end-systole (LADs), left atrium to aortic root (LA/Ao), left ventricular ejection fraction (LVEF), and fractional shortening (FS). All echocardiographic data were recorded and analyzed by a board-certified cardiologist in accordance with the standards of the American Society of Echocardiography and the ACVIM Cardiology Specialty guidelines.[17,18]

Echocardiographic variables were analyzed using a generalized mixed regression model of the form:

$$Y \sim \beta_0 + s(Time) + \beta_1 \text{ Affected} + \beta_2 \text{ Affected} * \text{Time} + \text{e}$$

where Y denotes one of the echocardiographic variables and s denotes a smooth function estimated from the data. Thus, the effects of time are modeled non-parametrically. The covariance structure within each dog was modeled as a continuous autoregressive process of order 1 (i.e., CAR1). Maximum likelihood was used to fit the model. All computations were performed using the R statistical software system. For each of the variables, interest centers on testing whether $\beta_1 < 0$ and $\beta_2 < 0$. Note that $\beta_1 < 0$ corresponds to the situation in which affected dogs have significantly lower values of the given echocardiographic variable at the earliest time period, and $\beta_2 < 0$ corresponds to the situation in which affected dogs have worsening values of the given echocardiographic variable over time. GraphPad Prism 5 software was also used to analyze the variables by linear and non-linear regression.

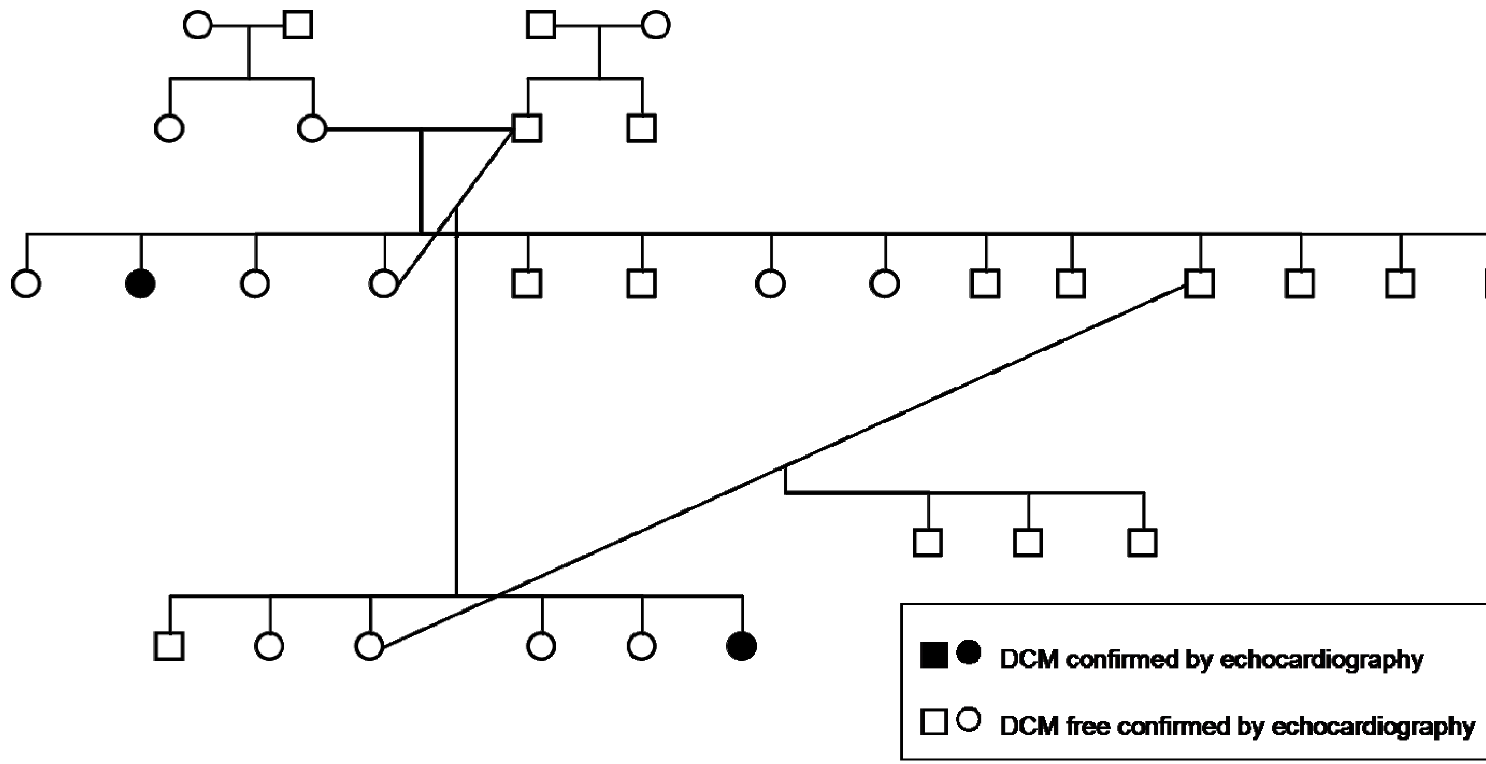

Figure 1 Pedigree showing the relationship between the analyzed Great Danes; squares – males, circles – females.

CHAPTER III

RESULTS

The twenty-two dogs were divided into two groups based on their health status. Nineteen dogs were labeled clinically normal, and the other three were labeled as having DCM. The health status of each animal was determined solely by echocardiographic examinations.

Interest centers on comparing the echocardiographic variables across affected and unaffected Great Danes over a period up to 300 days. Four variables were chosen for analysis based on their use as predictive indicators of DCM. Those echocardiographic variables chosen were LVDd, LVDs, LA/Ao, and %FS. Each variable was then analyzed using a generalized additive mixed regression model. The results for the four variables are as followed: (1) There is no statistically significant evidence that $\beta_1 < 0$ (p-value = 0.279/2) which corresponds to the situation in which affected dogs have significantly lower values of LVDd. There is statistically significant evidence that $\beta_2 < 0$ (p-value < 0.001) corresponds to the situation in which affected dogs have worsening values of LVDd over time. (2) There is statistically significant evidence that $\beta_1 < 0$ (p-value = 0.0160/2) which corresponds to the situation in which affected dogs have significantly lower values of left ventricular diameter in systole (LVDs) at the earliest time period. There is statistically significant evidence that $\beta_2 < 0$ (p-value < 0.001) corresponds to the situation in which affected dogs have worsening values of LVDs over time. (3) There is statistically significant evidence that $\beta_1 < 0$ (p-value = 0.0656/2) which corresponds to the situation in which affected dogs have significantly lower values of LA/Ao at the earliest time period. There is statistically significant evidence that $\beta_2 < 0$ (p-value < 0.001) corresponds to the situation in which affected dogs have worsening values of LA/Ao over time. (4) There is statistically significant evidence that $\beta_1 < 0$ (p-value = 0.00107/2) which corresponds to the situation in which affected dogs have significantly lower values of fractional shortening at the earliest time period. There is statistically significant evidence that $\beta_2 < 0$ (p-value = 0.07523/2) corresponds to the situation in which affected dogs have worsening values of fractional shortening over time. Figures 2-5 display the nonparametric estimate of the time effect for the data on each of the four variables.

Analysis of linear and non-linear regression was also used to assess the relationship between echocardiographic parameters of the two groups.[19] R^2 values were calculated to help determine the best fit line (Table 1). Significant differences were observed when comparing the two groups. Several echocardiographic values were increased or decreased for the dogs with DCM when compared to the normal dogs. The Great Danes with DCM showed increased values for left ventricular diameter at end-diastole and systole, left atrial diameter at end-systole, and LA/Ao ratio (Fig. 6, 7, 8). They also showed decreased values for IVS at end-diastole and systole, LVEF, and percent FS (Fig. 9). No changes were observed between the two groups for LVPW at end-diastole and systole or aortic diameter (Ao).

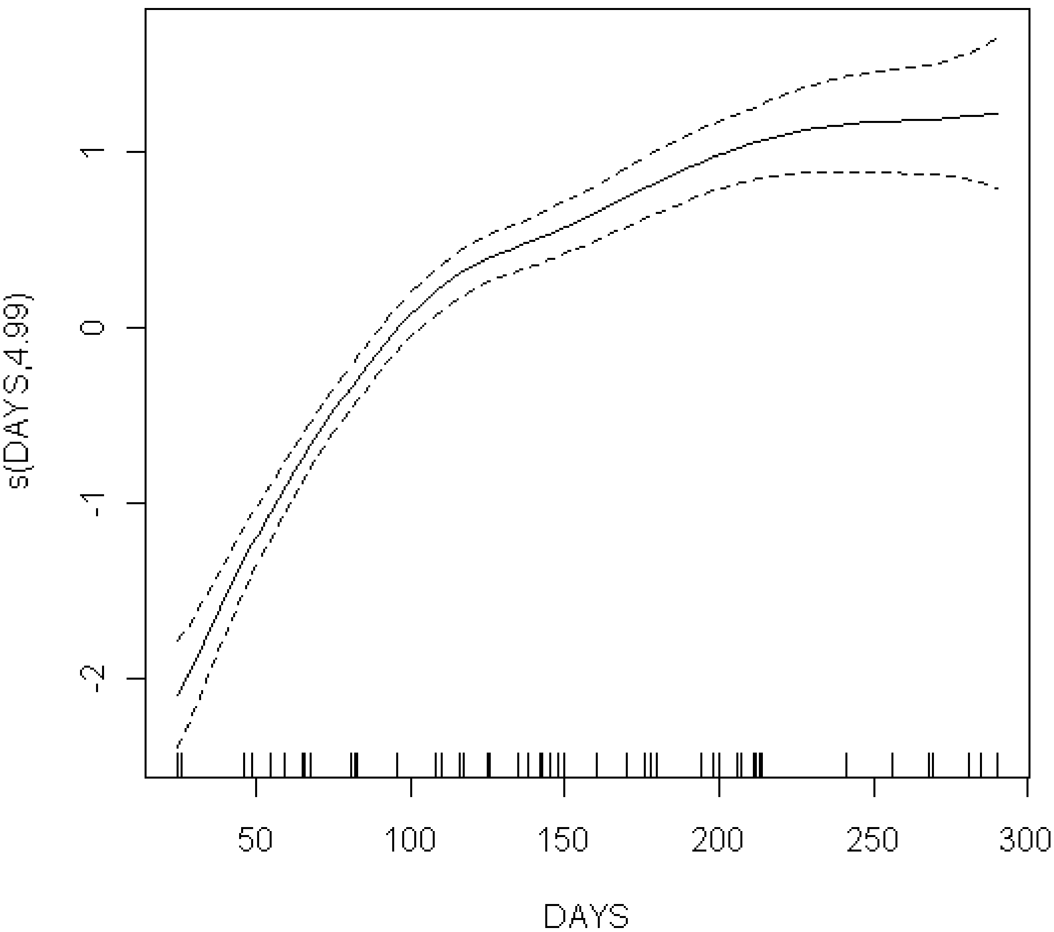

Figure 2 Nonparametric estimate of the time effect on left ventricular diameter at end-diastole.

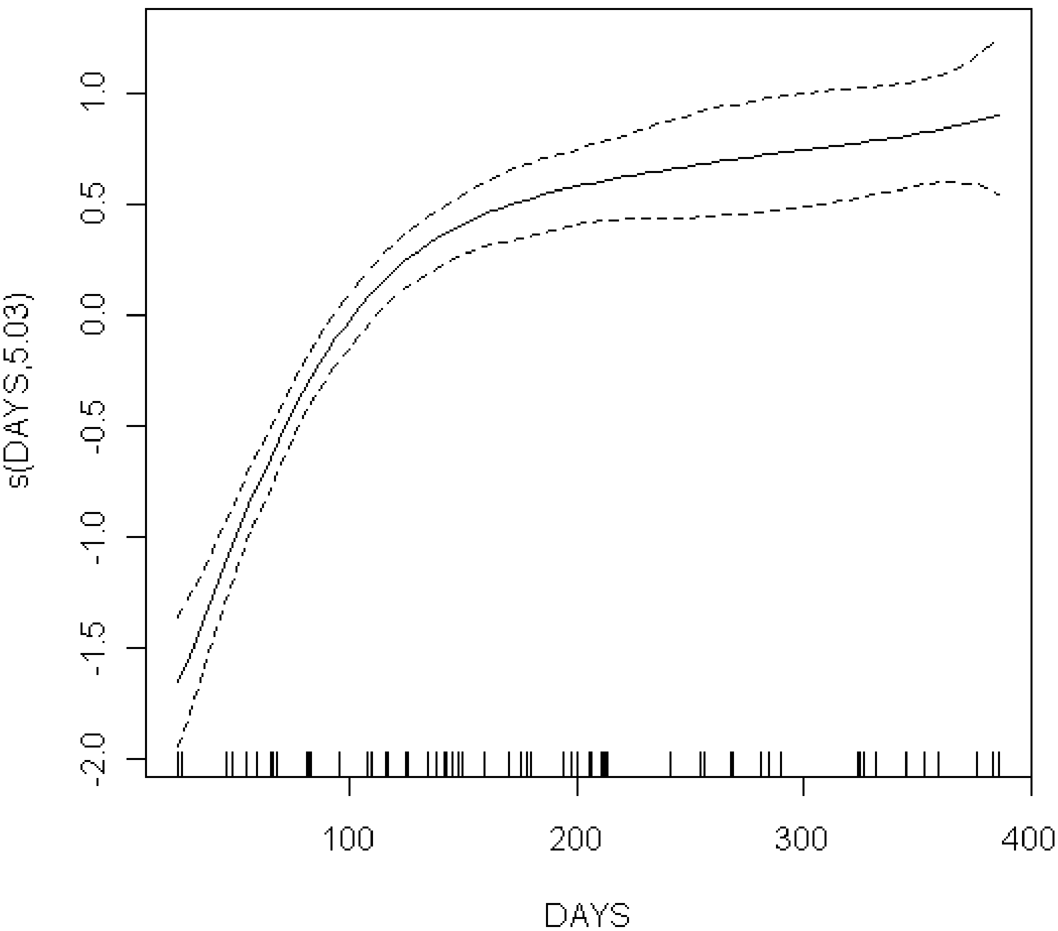

Figure 3 Nonparametric estimate of the time effect on left ventricular diameter at end-systole.

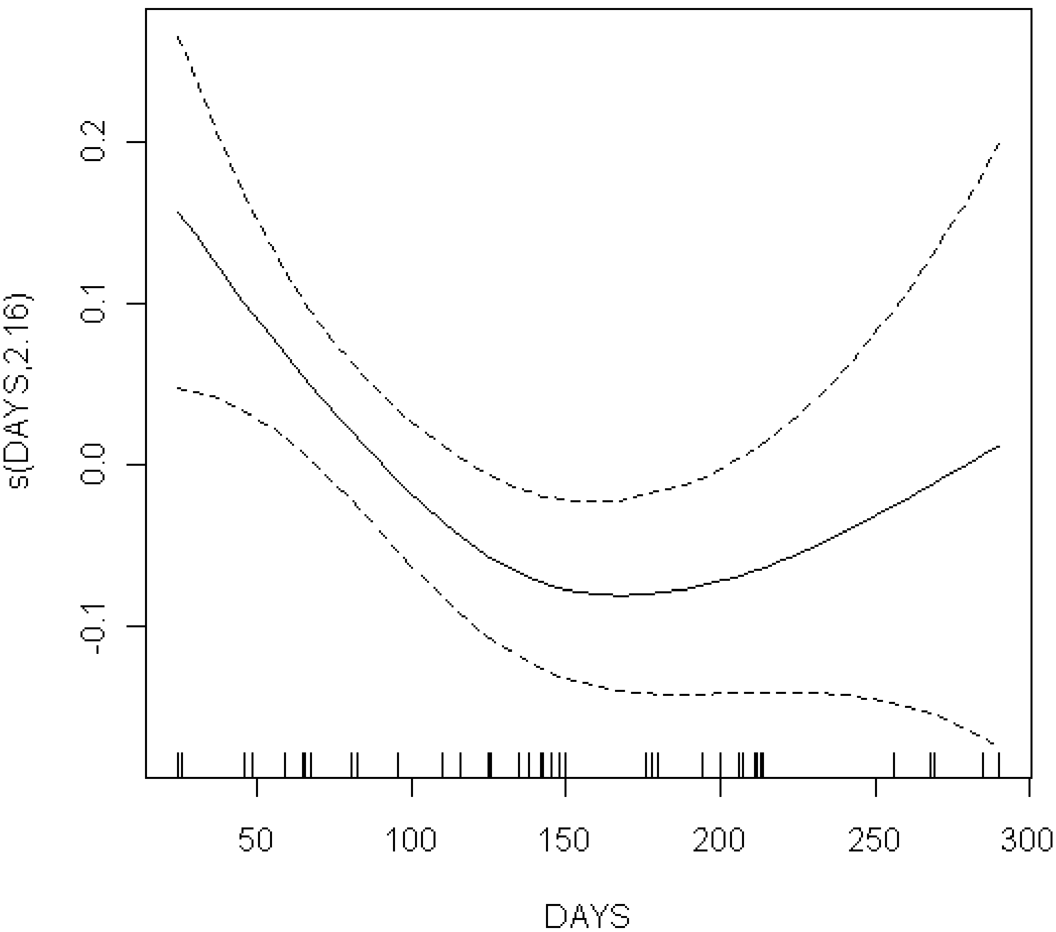

Figure 4 Nonparametric estimate of the time effect on the ratio between left atrial diameter at end-systole and aortic diameter.

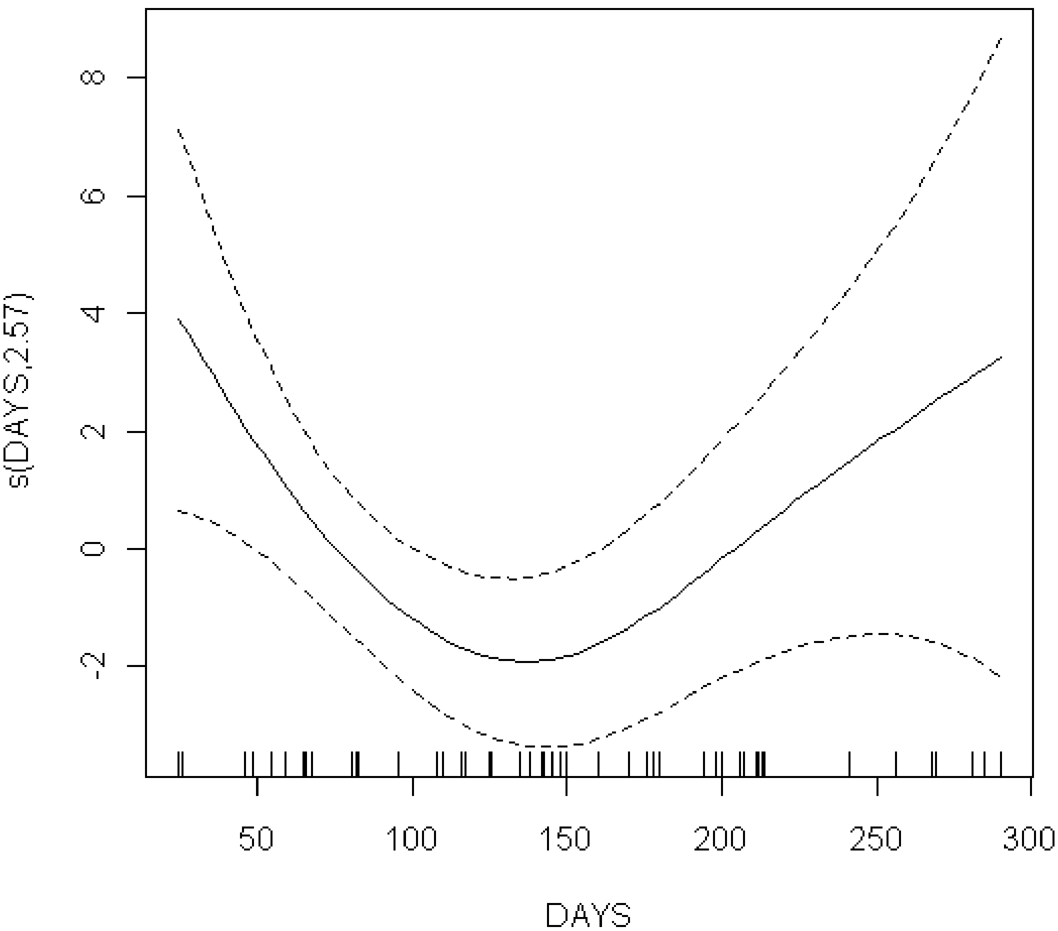

Figure 5 Nonparametric estimate of the time effect on the percent fractional shortening.

Table 1 R^2 Values		
	Normal (n = 19)	DCM (n = 3)
LVDd	0.8068	0.9435
LVDs	0.7510	0.9384
LVPWd	0.6776	0.8789
LVPWs	0.7241	0.6290
IVSd	0.5111	0.3456
IVSs	0.6714	0.2535
Ao	0.7751	0.7492
LADs	0.6893	0.8672
LA/Ao	0.0123	0.5141
LVEF	0.0001	0.7355
% FS	0.0024	0.8015

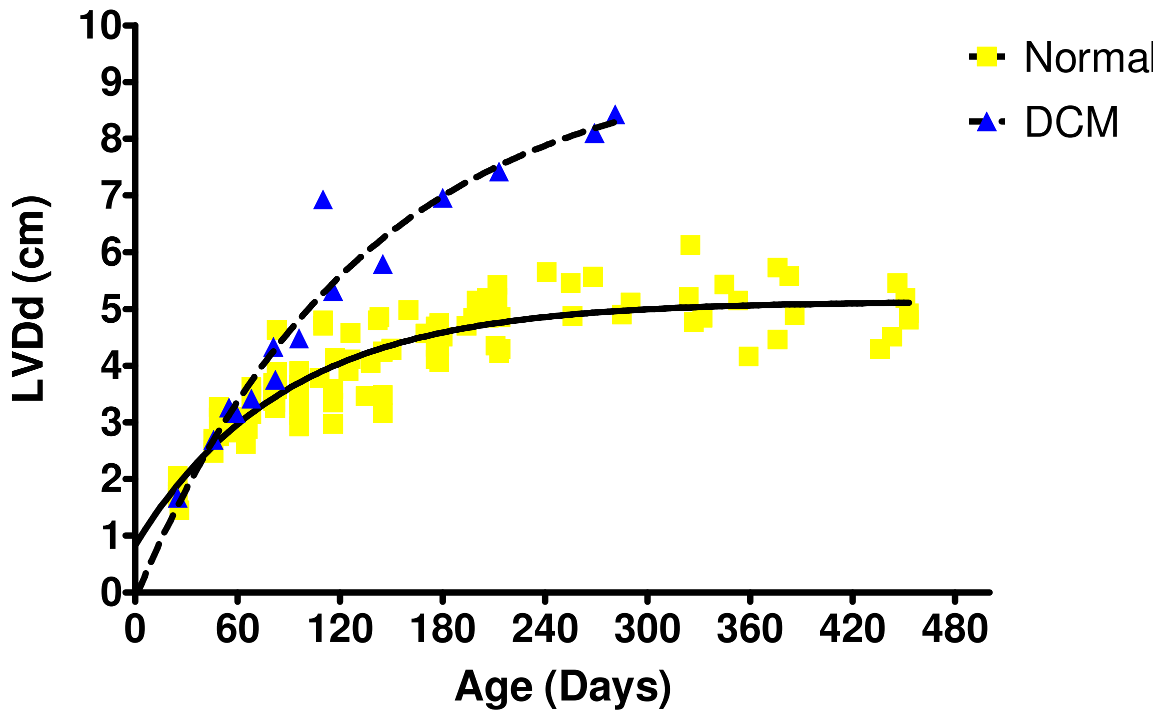

Figure 6 Non-linear graphs depicting left ventricular diameter at end-diastole of nineteen clinically normal Great Danes versus three Great Danes with dilated cardiomyopathy.

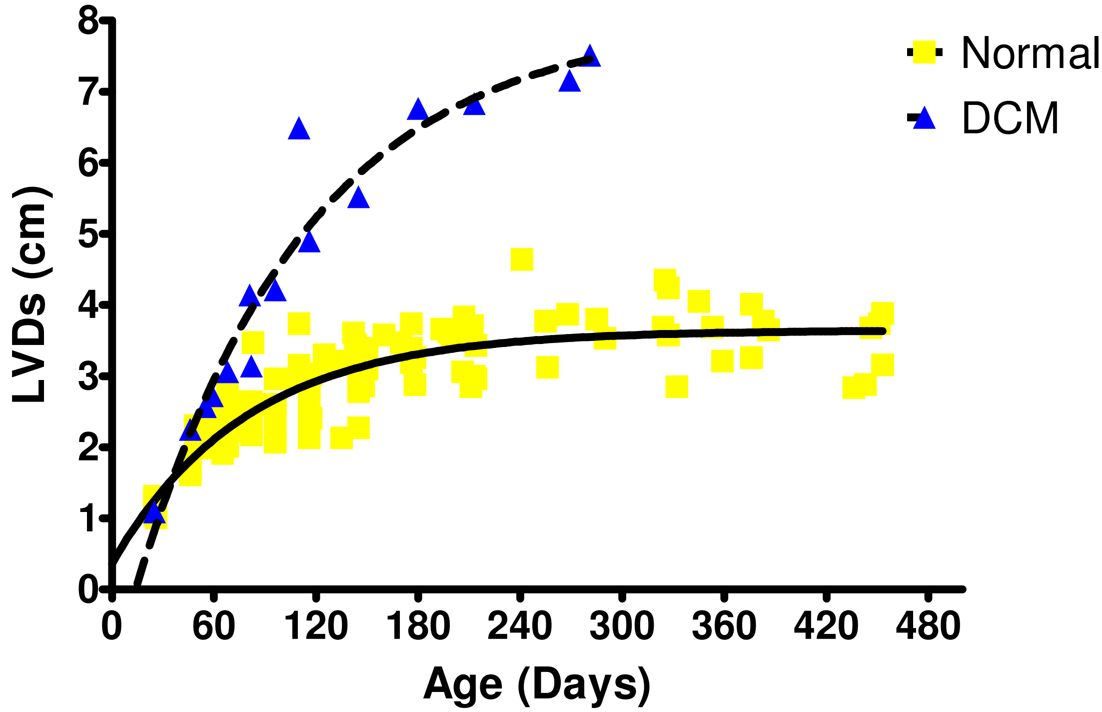

Figure 7 Non-linear graphs depicting left ventricular diameter at end-systole of nineteen clinically normal Great Danes versus three Great Danes with dilated cardiomyopathy.

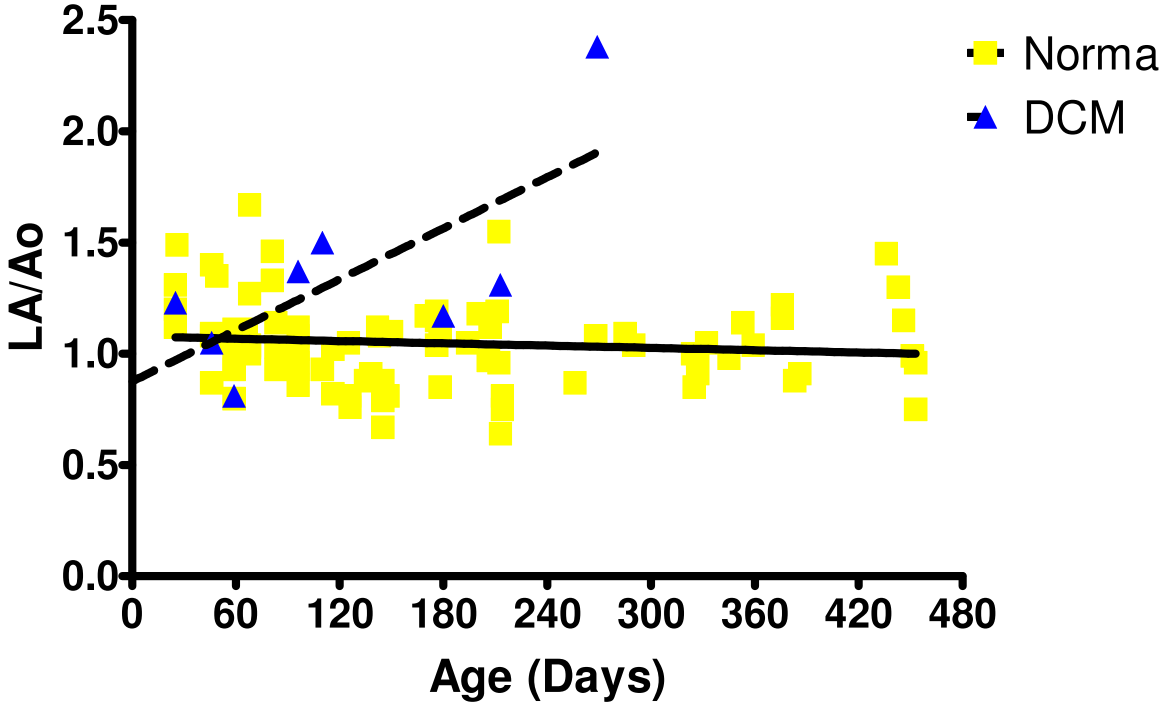

Figure 8 Linear graphs depicting the ratio between left atrial diameter at end-systole and aortic diameter of nineteen clinically normal Great Danes versus three Great Danes with dilated cardiomyopathy.

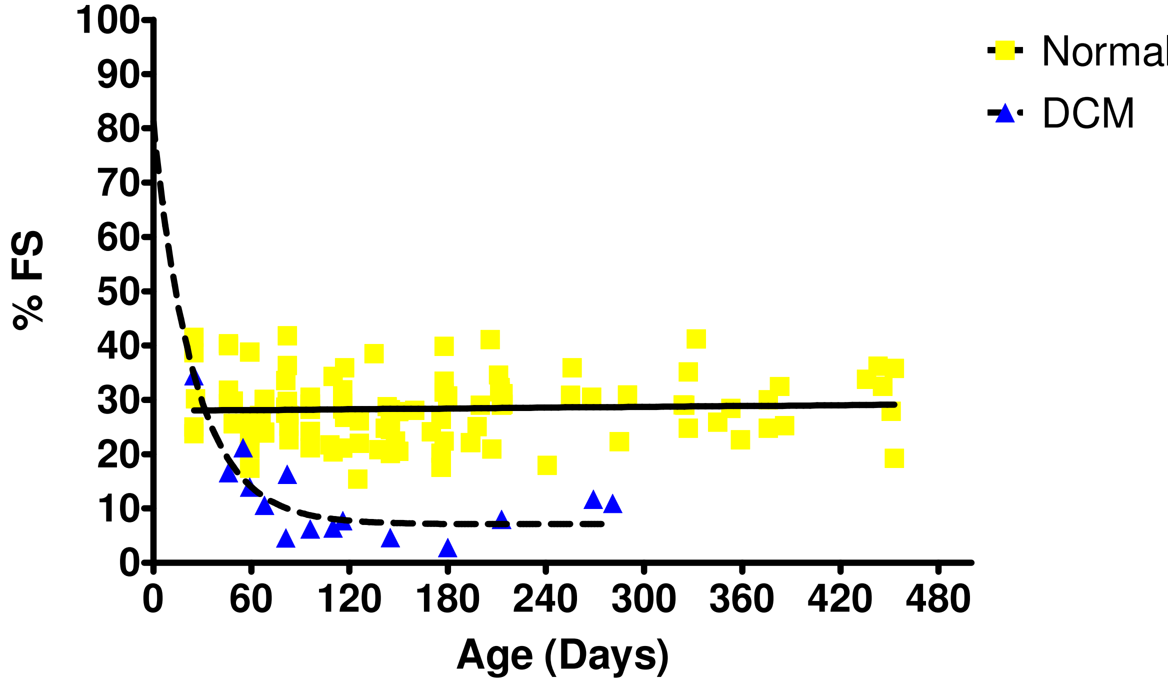

Figure 9 Linear and non-linear graphs depicting percent fractional shortening of nineteen clinically normal Great Danes versus three Great Danes with dilated cardiomyopathy.

CHAPTER IV

DISCUSSION

DCM is a myocardial disease that can cause chamber dilation, wall thinning, wall motion abnormalities, and a decrease in systolic ejection phase indices.[11] It is one of the leading causes of cardiovascular mortality in dogs and affects several breeds.[8]

Echocardiography plays an integral role in evaluating cardiac diseases including DCM. For it to be used as a successful diagnostic tool, knowledge of normal reference values is needed.[20] Normal echocardiographic parameters have been published for several breeds. Some of the breeds for which published parameters are available include the Beagle, Miniature Poodle, Pembroke Welsh Corgi, Afghan Hound, Golden Retriever, English Cocker Spaniel, Newfoundland, Irish Wolfhound, Great Dane, and Spanish Mastiff.[16,21-24] Although breed specific parameters have been established very few studies use serial echocardiographic evaluation.[14]

Using serial echocardiography we were able to document the progressive echocardiographic changes seen in normal Great Danes and Great Danes with DCM. The three Great Danes with DCM had significantly increasing values of left ventricular and left atrial diameter, and a lower fractional shortening over time. They also showed thinning of the interventricular septum and decreased ejection fraction. Additionally, the affected dogs had significantly lower values of LVDs, LA/Ao, and %FS at the earliest time period when compared to the normal dogs. This raises the question of whether these values are early indicators of future systolic dysfunction.

In a study of giant breed dogs, normal echocardiographic values were recorded in Newfoundlands, Irish Wolfhounds, and Great Danes. The published values of the fifteen normal Great Danes in that study were compared with the data we collected on nineteen normal Great Danes. The data from the eleven echocardiographic parameters we measured fit the ranges published by Koch, et al (Figs. 10-13).[16]

One major potential limitation of this study is the sample population. The data was collected from a small colony of Great Danes that have been bred to known carriers of DCM. All though many of the dogs used for the study show no clinical symptoms, they could potentially develop the disease later in life. The sample, composed of twenty-two animals, is most likely not a representative of the Great Dane population.

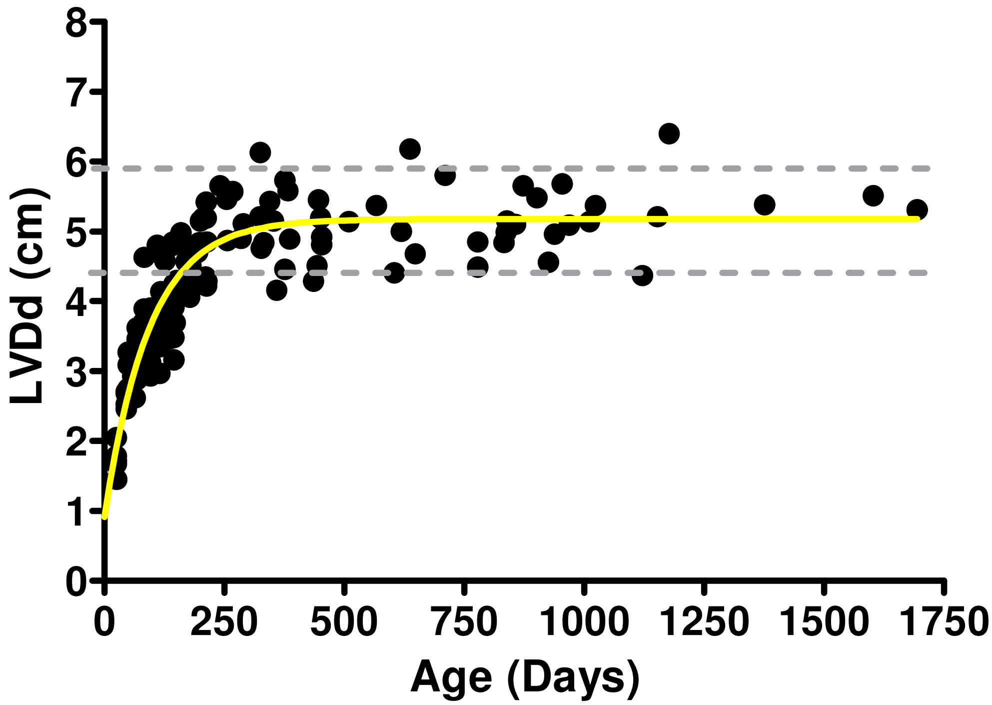

Figure 10 Non-linear graph depicting left ventricular diameter at end-diastole of nineteen clinically normal Great Danes. The dotted lines represent a range (4.4 – 5.9) determined by Koch et al. in fifteen clinically normal Great Danes.

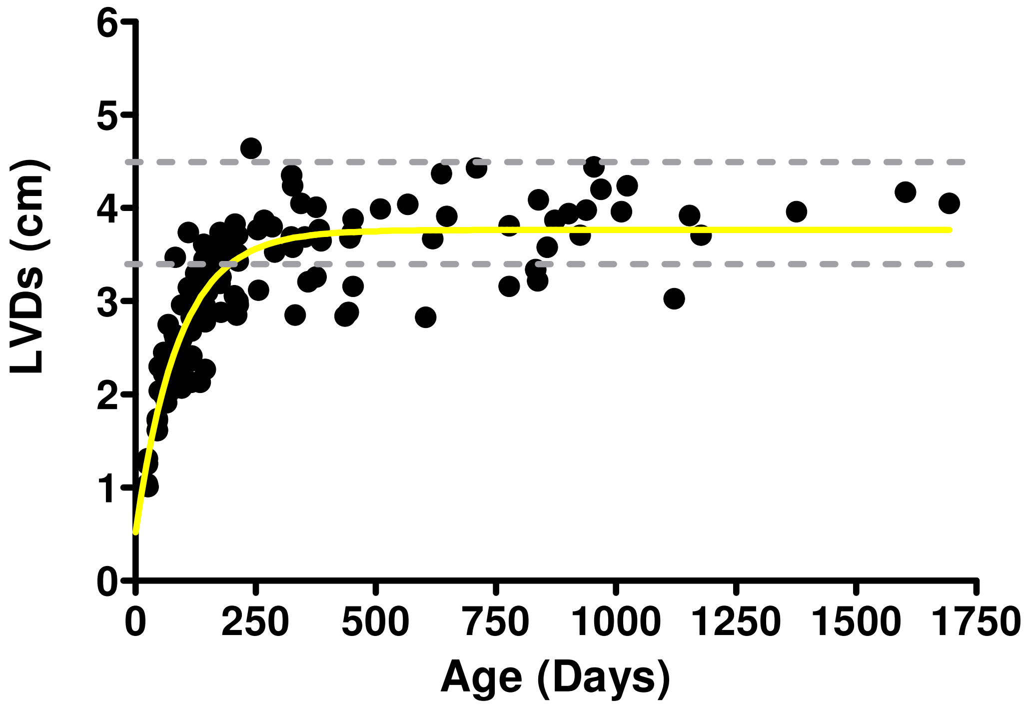

Figure 11 Non-linear graph depicting left ventricular diameter at end-systole of nineteen clinically normal Great Danes. The dotted lines represent a range (3.4 – 4.5) determined by Koch et al. in fifteen clinically normal Great Danes.

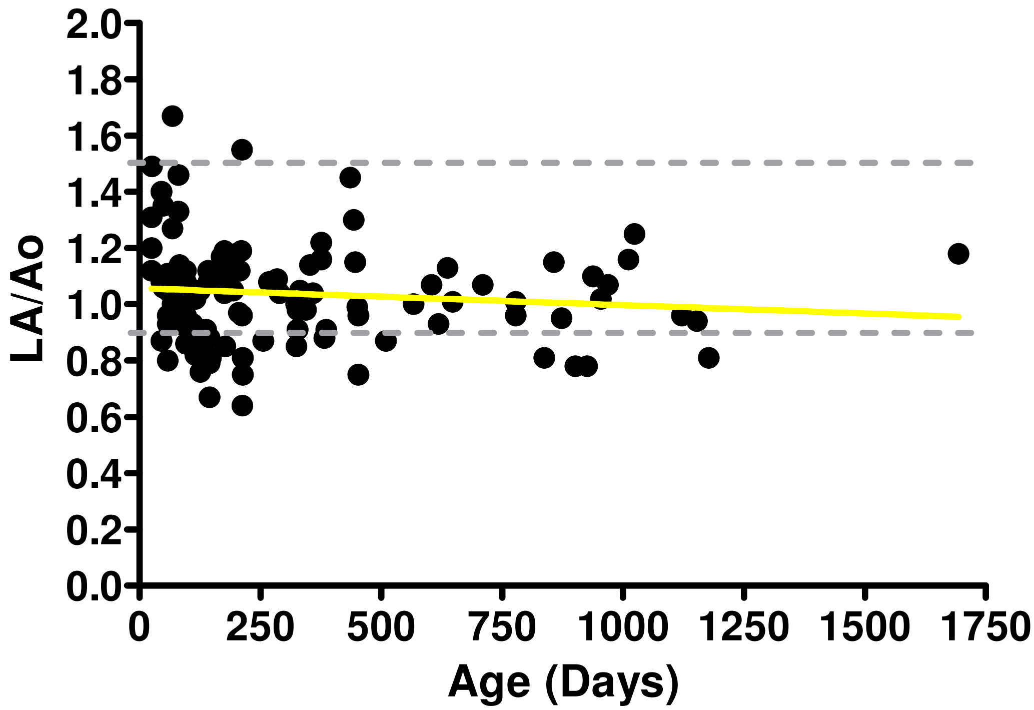

Figure 12 Linear graph depicting the ratio between left atrial diameter at end-systole and aortic diameter of nineteen clinically normal Great Danes. The dotted lines represent a range (0.9 – 1.5) determined by Koch et al. in fifteen clinically normal Great Danes.

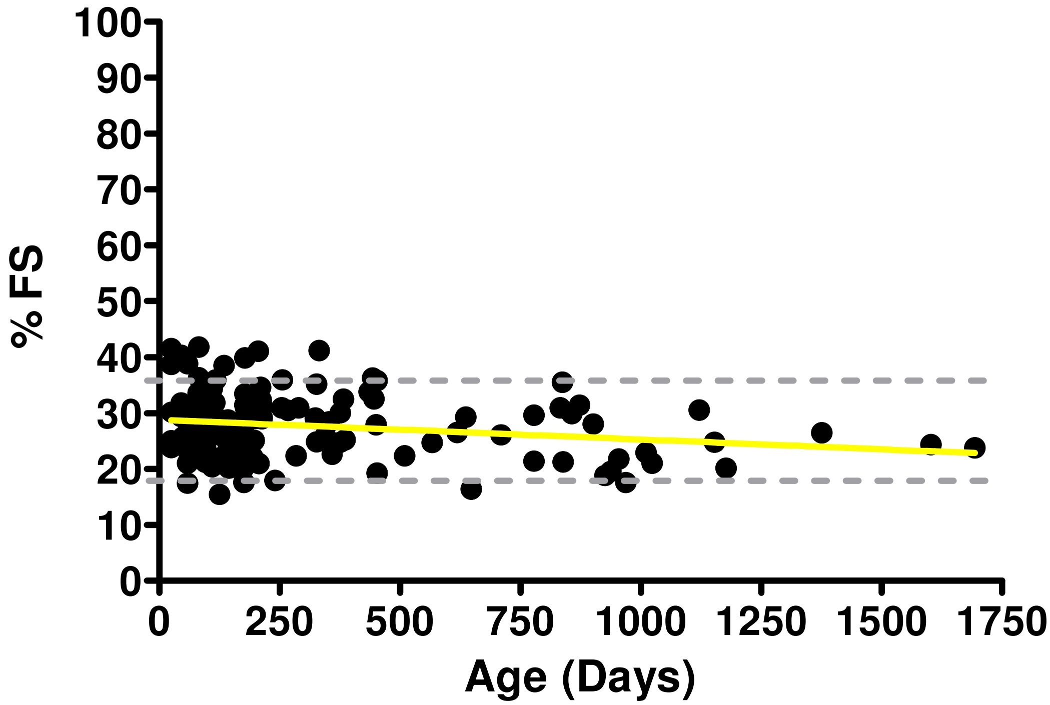

Figure 13 Linear graph depicting percent fractional shortening of nineteen clinically normal Great Danes. The dotted lines represent a range (18 – 36) determined by Koch et al. in fifteen clinically normal Great Danes.

CHAPTER V

CONCLUSION

DCM is an inevitably fatal disease that affects many canine breeds.[8] In this study we documented the progressive echocardiographic changes seen in both clinically normal Great Danes and Great Danes with DCM by using serial examinations. Several authors have commented on the use of serial echocardiography for an earlier diagnosis of preclinical disease, and for development of breed specific parameters.[14,25-27] Although linear studies take several years to complete, the data collected could lead to more specific parameters, and a more specific age recommendation for baseline screening.

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